PREDICTING BIOLOGICAL WARFARE AGENT DETECTOR PERFORMANCE

by

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DEDICATION

This is dedicated to my father Master Sergeant Clark Lee Holman who was killed in action in Korea, July 1950.

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I would like to thank my lovely wife Debi for her understanding and encouragement while I worked on this dissertation. I am very grateful to the chair of my dissertation committee, Andrew Loerch, and Donald Gantz for their contributions to this dissertation. I would also like to thank Chuck Jennings, Carl Russell, Russell Bartholomew, Doug Anderson, and Chee Chan from the whole system live agent test and evaluation team and data authentication group.

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LIST OF ABBREVIATIONS

ABT – Ambient Breeze Tunnel
ALO – Agent-like Organism
ASEC – Aerosol Simulant Exposure Chamber

BAWS – Biological Agent Warning System
BL-1 – Bio-level safety one
BL-3 – Bio-level safety three
BT – *Bacillus Thuringiensis*
BWA – Biological Warfare Agent
BG – *Bacillus subtilis*

CAC – Containment Aerosol Chamber

EH – *Erwinia herbicola*

FTS – Fluid Transfer System
JBPDS – Joint Biological Point Detection System
JBSDS – Joint Biological Agent Detection System
JCAD – Joint Chenical Agent Detector
JSLSCAD – Joint Service Lightweight Standoff Chemical Agent Detector

L – Likelihood function
l – log of the Likelihood function
LE – BWA code

MOT&E – Multi-service Operational Test and Evaluation
MS2 – Male Stereotype 2

N – BWA code
NU – BWA code

SAS – Statistical Analysis System

T&E – Test and Evaluation

XR – BWA code

ABSTRACT

PREDICTING BIOLOGICAL WARFARE AGENT DETECTOR PERFORMANCE

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Biological warfare agents (BWAs) are inherently dangerous. United States of America public law forbids the release of biological warfare agent into the environment. The test and evaluation community desires realistic open air field tests, but does not want to risk harming test participants or the general public. Neither do they want to break the law by releasing actual BWAs during open air field tests.

To test biological defense systems in the field, the test and evaluation community releases relatively harmless substances known as simulants. It is highly desirable that the biological warfare system under test performs identically with the simulant as it does with real biological warfare agent. Unfortunately this never occurs.

A new class of simulants known as Agent-Like Organism (ALO) has been developed. An ALO is phylogenetically closely related to its corresponding biological warfare agent. A vaccine strain is an example of ALO.

The purpose of this dissertation is twofold: to determine if killed or inactivated

ALOs are acceptable simulants for a biological point detection system that uses particle fluorescence and immunoassay technology; and to develop a model based on logistic regression to relate detector simulant performance to detector performance with biological warfare agent.

Data for this analysis was obtained from Dugway Proving Ground, Utah. The data set consisted of 2,717 Joint Biological Point Detector System (JBPDS) challenges. The system was challenged with either BWA or ALO simulant. All BWA challenges occurred in a Bio-Level 3 (BL-3) facility. Data was analyzed using logistic regression. The determination as to the acceptability of the ALOs as simulants was based on both statistical analysis and judgment on the impact of differences on the performance in an open air field test.

Results are given that show the acceptability of various simulants for particular BWAs with respect to both detection and identification. The simple models examined in this dissertation do not adequately explain the variability that occurs in field open air releases. As a result of unexplained variability in detector performance during open air field releases, even the best predictive model is of minimal utility in predicting detector performance.

The best predictor of biological warfare agent detector performance is field trials with killed ALOs. A possible method to improve the acceptability of Killed N ALO and Killed NU ALO as identification simulants is discussed.

Chapter 1 Introduction

Statement of the Problem

Biological warfare agents (BWAs) are inherently dangerous. United States of America public law forbids the release of biological warfare agent into the environment. The test and evaluation community desire realistic field tests, but does not want to risk harming test participants or the general public. Neither do they want to break the law by releasing actual BWAs during field tests.

To test biological defense systems in the field, the test and evaluation community releases relatively harmless substances known as simulants. It is highly desirable that the biological warfare system under test performs identically with the simulant as it does with real biological warfare agent. Unfortunately this never occurs.

The purpose of this dissertation is twofold:

1. to determine if killed or inactivated Agent-Like Organism (ALO) are good simulants for a biological point detection systems that use particle fluorescence and immunoassay technology and second
2. to determine if a simple model can be developed to relate detector simulant performance to detector performance with biological warfare agent and if that model is a reasonable analytical construct for use in evaluation.

ALOs are often vaccine strains. They are discussed in chapter two. Fluorescence occurs when a substance is exposed to electromagnetic waves and emits electromagnetic waves of a different wave length than it was exposed to. An immunoassay is based on the combination of an antibody with an antigen. Antibodies are produced by living organisms as part of the defense against foreign substances, known as antigens. The immunoassay contains antibodies that will bind to an antigen of a specific BWA.

Results from this dissertation will be used to evaluate the Joint Biological Point Detection System (JBPDS) and other chemical and biological defense systems. The department of defense plans on procuring 2,165 JBPDSs at a total program cost of over $1.1 Billion. They will be fielded to the Army, Marines, Navy, and the Air Force. They will be the backbone of tactical biological defense for the armed forces. For the Army, they represent the third generation of biological warfare detection system. They will also be employed in defense of our homeland.

Joint Biological Point Detection System (JBPDS)

The JBPDS is an integrated system designed to automatically detect and presumptively identify the presence of BWA aerosols to allow appropriate defensive measures and minimize casualties. The JBPDS provides an audible and visual indication of the presence of BWAs and displays readouts of their identification. It also has the capability of producing a sample for transport to a designated laboratory. The designated laboratory will provide conformation and verification of a biological attack and the definitive identification of the agent.

JBPDS functions in a two step process. The first step is detection. The second step is identification. The detector is continually sensing the environment. The

identification step is turned on only after detection has occurred. The detection process occurs in the Biological Agent Warning System (BAWS) which is a line-replaceable subsystem. The identification process occurs in another line-replaceable subsystem known as the identifier.

The JBPDS is composed of five main line-replaceable units and depicted in figure 1-1. They are the BAWS, the collector, the Fluid Transfer System (FTS), the identifier, and the controller assembly.

- The BAWS is trigger and detector. Environmental air is sucked in to the BAWS, which is continually sensing the environment. The BAWS compares the number and intensity of biological particles to the profile of biological particles which it has "seen" in the resent past. Increases in the number or intensity of biological particles may result in detection. Fluorescence from two to ten micron size particles is the principle means by which the BAWS detects biological particles. Fluorescence occurs when a substance is exposed to electromagnetic waves and emits electromagnetic waves of a different wave length than it was exposed to. The detection will automatically turn on the collector.
- The collector is a wetted-wall cyclone collector. It collects a sample from the air which is captured in swirling buffer solution and transfers it to the FTS.
- The FTS is a series of tubes that transports the liquid sample to a reservoir. This reservoir fills the identifier and sample evacuation vials.
- The identifier uses immunoassay strips that are specific to its complementary antibody. Multiple strips are contained in a carrier. Carriers are formatted with differing types of specific (antibody) assay strips for identification of the BWA.

The identifier uses cartridges in a rack and stack method. Multiple carriers are indexed in the cartridges. When the identifier receives the appropriate signal, a liquid sample from the FTS is automatically injected onto the assay strips. The strips, housed in a carrier, have identification markers that appear when a liquid sample of the BWA is inoculated onto the matching antibody strip. The control markers appear each time liquid is inoculated onto the strip. An optical reader of the carrier strips provides a means for identification.

- The controller assembly provides computer control of the various JBPDS subsystem during operations.

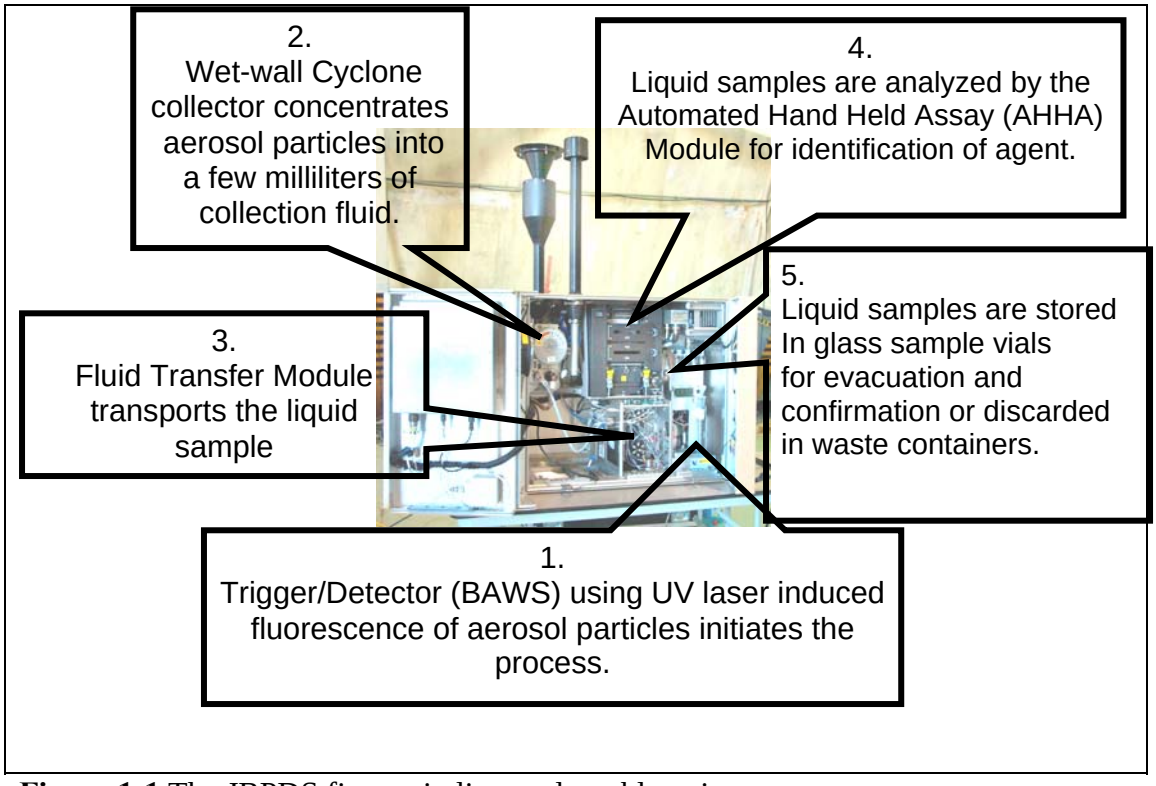


Figure 1-1 The JBPDS five main line-replaceable units.

Weapon

A biological warfare weapon basically consists of three types of components. They are the delivery system, additives, and biological warfare agents. Delivery systems include missiles, bombs, aerosol spraying devises, envelopes, and salad bars. Additives are substances that enhance the effectiveness of the delivery and the agent. Agents are discussed in the next section. For realistic field tests, the tester must also use realistic delivery methods and additives. This dissertation focuses on simulating or replicating the agent.

Agents

Good summaries of biological warfare agents are found in Kortepeter et al., (2001) and Weinstein and Alibek (2003).

The most common biological warfare agents are bacteria, viruses, and toxins.

Bacterial agents include *Bacillus anthracis* which is the causative agent of anthrax, *Brucella suis* and *Brucella melittensis* which are the causative agents of brucellosis, *Burkholderia mallei* which is the causative agent of glanders, *Burkholderia pseudomallei* which is the causative agent of meliodosis, *Yersinia pestis* which is the causative agent of plague, *Coxiella burnetii* which is the causative rickettsia-like agent of Q fever, and *Francisella tularensis* which is the causative agent of Tularemia. *B. anthracis* is a spore forming gram-positive bacillus. All other agents listed are non-spore forming gram-negative bacteria. *B. suis* and *B. melittensis* are coccobacilli. *B. mallei*, *B. pseudomallei*, *F. tularensis*, and *Y. pestis* are bacillus.

The bacteria *B. anthracis* is the causative agent of cutaneous anthrax, gastrointestinal anthrax, oral-pharyngeal anthrax, and inhalational anthrax. The

incubational period for cutaneous anthrax is 1 to 12 days, for both gastrointestinal and oral-pharyngeal anthrax is 3 to 5 days, and for inhalational is 1 to 13 days. The mortality rate, without treatment is: 40 to 100% for inhalational anthrax, 25 to 100% for both oral-pharyngeal and gastrointestinal anthrax, and about 20% for cutaneous anthrax. If treatment with an antibiotic such as ciprofloxacin or doxycycline starts within two days following infection mortality can be greatly reduced. Cutaneous anthrax results in skin lesions and release of toxins into the body. Both oral-pharyngeal and gastrointestinal anthrax will result in fever, nausea, ulcers, vomiting, anorexia, and acute abdominal pain. Inhalational anthrax will result in malaise, flu-like symptoms, high fever, extreme fatigue, sever respiratory distress, cyanosis, septicemia, and death. Anthrax spores are resistant to chemical disinfectant, heat, and environmental factors that easily kill vegetative bacteria. *B. anthracis* has been used as a biological weapon. In 2001 small amounts of *B. anthracis* were distributed in letters. This resulted in 22 cases of infection.

Tularemia which is also known as rabbit fever or deerfly fever is caused by the vegetative bacteria *F. tularensis*. Unlike most other pathogenic bacteria, *F. tularensis* is an intracellular parasite. During phagocytosis by the immune system it enters a host cell but evades intracellular killing. In nature *F. tularensis* most commonly infects lagomorphs (rabbits or hares) and rodents although it may also infect many other mammal species and even some birds. Tularemia can be transmitted by arthropod bites, direct contact with infected tissues, inhalation, or ingesting infected meat or contaminated water. Person to person transmission of this disease has not been documented. The incubation period is 2 to 5 days. Symptoms for the pneumonic clinical form include fever, headache, muscle pain, shortness of breath and difficulty breathing, cough, and

pleural pain. Fatality for untreated patients is between 7 and 50%. Antibiotic treatment can reduce fatalities to less than 2%. *F. tularensis* could be delivered as a biological warfare weapon by an aerosol, contaminated food, or by releasing infected vectors. *F. tularensis* was likely used as a biological warfare weapon by the Japanese in Manchuria between 1932 and 1945.

Plague also known as "black death" is caused by *Y. pestis*. There are four main primary clinical forms of plague. They are bubonic, septicemic, pneumonic, and pharyngeal. Rodents, especially rats are a natural reservoir for *Y. pestis*. Plague is typically transmitted to other rodents and humans by the flea vector. The incubation period for bubonic plague is 2 to 6 days and for pneumonic is between 1 to 3 days. Symptoms of bubonic plague include fever, chills, weakness, and the development of swollen tender lymph nodes. Symptoms of pneumonic plague include bronchopneumonia, chest pain, dyspnea, cough, and hemoptysis. Without treatment fatality rate for bubonic plague is between 50 to 90%. Without treatment pneumonic plague typically results in 100% fatalities. With antibiotic treatment, the fatality rate can be reduced to 5% for bubonic plague and 20% for pneumonic plague. The most common clinical form following an attack using vectors would be bubonic. The most common clinical form following an aerosol attack would be pneumonic. In the 1346 siege of Caffa on the Crimean peninsula in the Black Sea, the Tartars hurled plague infected bodies over the city's defensive walls to infect the city residence. *Y. pestis* was used as a biological warfare weapon by the Japanese in Manchuria between 1932 and 1945.

In nature, brucellosis can be caused by *B. suis, B. melittensis, Brucella abortus* and *Brucella canis.* However, only *B. suis* and *B. melittensis* are expected threat

biological warfare agents. Like *F. tularensis*, the *Brucella* species are intracellular parasites. *B. suis* primarily infects pigs and *B. melittensis* primarily infects sheep and goats. Brucellosis is naturally transmitted by direct contact or ingestion of unpasteurized dairy products. Human to human transmission is rare. The incubation period is about 2 weeks. Brucellosis is a debilitating and prolonged disease, but it is rarely fatal. Symptoms for the acute clinical form include fever, sweats, malaise, anorexia, headache, myalgia, and back pain. Brucellosis is treated with antibiotics for 6 weeks. The primary biological warfare threat is from an aerosolized attack.

Glanders is a highly contagious disease. It primarily infects equids such as horses, mules, and donkeys. In nature transmission to humans is rare. *B. mallei* which is the cause of glanders is found only in infected mammalian hosts. The incubation period is from 2 to 14 days. The untreated mortality rate is about 50%. Symptoms of the pulmonary clinical form include fever, rigors, sweats, cough, myalgia, chest pain, photophobia, lacrimation, diarrhea, and tachycardia. Treatment is the administration of antibiotics for 60 to 150 days. The primary threat is aerosolized agent. *B. mallei* may have been used as a biological weapon against cavalry horses during World War I.

Meliodosis also known as Whitmore disease is cased by the vegetative bacteria *B. pseudomallei*. This bacterium is commonly found in tropical soil and water. Infection can be caused by inhalation of dust containing *B. pseudomallei*, drinking contaminated water, or by contact with contaminated soil. Transmission may also occur from an infected person or animal. The incubation period is variable and may range from 2 days to several years. Symptoms of the pulmonary clinical form include fever, headache, anorexia, muscle soreness, chest pain, and cough. Meliodosis has a 90% mortality rate.

Death usually occurs within 2 days after the onset of symptoms. A bio-attack is most apt to be with aerosolized agent.

Viral agents include *Variola major* which is the causative agents of smallpox, *Orthopoxvirus* which is the causative agents of monkeypox, Venezuelan equine encephalitis (VEE) virus, Ebola virus, Marburg virus, and Rift Valley fever virus. *Variola major* and *Orthopoxvirus* are poxviridae. Poxviridae are large viruses with double stranded DNA, and an envelope derived from the host cell membrane. VEE is in the family togaviridae. Togaviridae are single stranded positive-sense RNA viruses with a lipid and glycol-protein envelope. VEE is transmitted by mosquitoes. Ebola and Marburg viruses are filoviridae. Filoviridae are single stranded, negative-sense RNA viruses with a lipid envelope. They are filamentous. Rift Valley fever virus is a member of the bunyaviridae family. Bunyaviridae are single-stranded negative sense viruses with an envelope.

Smallpox is a highly communicable viral disease. It affects only humans. About 30% of smallpox cases will result in death. The incubation time is 3 to 19 days. There is no effective medical cure. In 1980 the World Health Assembly declared smallpox eradicated. It was eradicated by an aggressive vaccination program. Since its eradication, the vaccination program has stopped. Live *Variola major* is stored in both Russia and the United States of America. It is likely or at least possible that several countries possess *Variola major* biological weapons. Smallpox attacks could occur as contamination in the food or water supply, contamination in various articles such as letters or clothes, or as an aerosol. As few as 5 to 10 viral particles can cause an infection. *Variola major* can survive up to 24 hours in the air. Smallpox has been used

as a biological weapon. From 1754 to 1767 the British Army distributed smallpox contaminated blankets to Native Americans.

Marburg and Ebola are hemorrhagic virus with high mortality rates. The mortality rate for Marburg is between 30 and 70%. The Mortality rate for Ebola is between 30 and 90%. The incubation period for Marburg is 3 to 12 days. The incubation period for Ebola is 2 to 21 days. Death may be caused by encephalitis or fulminant hepatitis, but is more commonly the result of pulmonary and gastrointestinal hemorrhage. There is no known cure for either Marburg or Ebola, although ribavirin is often administered.

Venezuelan equine encephalomyelitis (VEE) is a mosquito-born disease. It most commonly affects horses, donkeys, mules, and zebras. Humans can also contract the disease. There have been three large epidemics in Venezuela and Columbia. These epidemics resulted in 300,000 human infections and 2,000 deaths. As few as one viral partial can cause an infection. The incubation period is from 1 to 5 days. There is no cure for VEE.

Toxins include botulinum toxin, ricin, abrin, and Staphylococcal Enterotoxin B. All toxins are proteins and lack the prokaryotic cellular structure of bacteria and the complex virion structure.

Botulinum toxin is produced by the obligate anaerobe, spore forming bacteria *Clostidium botulinum.* The incubation period for this toxin is 12 to 36 hours. The classic diagnostic symptoms for botulism are flaccid skeletal muscle paralysis and bulbar palsies. Other symptoms include blurred vision, ptosis, collapse of the upper airways, and respiratory muscle paralysis. Medical management is by supportive care which includes

breathing assistance. Botulinum toxin has been used as a biological weapon by terrorist groups. The Japanese cult Aum Shinriky used botulinum toxin to attack a US Navel Base in 1990, Prince Naruhito's wedding in 1993, and Kasumigaseky subway station in 1995. Fortunately these three attacks failed to produce any casualties because the weapons were not prepared properly.

Ricin is produced from the beans of the caster plant *Ricinus communis*. Caster plants are grown world wide. This toxin blocks protein production. Symptoms include difficulty breathing, fever, cough, nausea, sweating, and tightness in the chest. Medical management is by supportive care which includes breathing assistance. It can cause death in 36 to 72 hours after exposure. Ricin could be distributed as a biological warfare weapon as an aerosol, in food, or as an injection.

Abrin is produced from the rosary pea plant *Abrus precatorius*. All parts of the plant contain the toxin abrin, but the seeds are the best souse for this toxin. Abrin is similar to ricin. Like ricin, abrin blocks protein synthesis. The symptoms, medical management, and use as a weapon of abrin are the same as ricin.

Staphylococcal enterotoxin B commonly called SEB is a toxin produced by the bacteria *Staphylococcus aureus*. Staphylococcal enterotoxins are the most common cause of food poisoning. *Staphylococcus aureus* can also cause pus-forming infections and toxic shock syndrome. Symptoms occur within 3 to 12 hours. Symptoms include fever, chills, headache, myalgia, diarrhea, vomiting, abdominal pain, and a nonproductive cough. It is incapacitating, but rarely lethal.

Classification Note

This dissertation is unclassified. Care has been taken not to include any classified information. Since detector performance against BWA is classified, no detector performance data or results are presented. Test of hypotheses will include comparing detector performance against agents to detector performance against killed agent, ALOs, and killed ALOs. But, no actual system performance data is included.

Chapter 2 Literature

The laboratory chamber is the only environment in which detector performance can be measured against live agent. According to the Defense Threat Reduction Agency, which is responsible for Department of Defense simulant research, the literature on predicting detector performance against actual agent in a field environment is sparse. This is true for both chemical warfare agent detectors and biological warfare agent detectors (Kaufman 2005).

This chapter will provide:

- a description of the chemical and biological test and evaluation paradigm, which provides motivation for the use of simulant,
- a comprehensive review of biological warfare simulant literature,
- a comprehensive review of agent simulant transformation literature, and
- a review of current Department of Defense basic research on simulants.

Chemical and Biological Warfare Test and Evaluation Paradigm

Chemical and Biological agents are inherently dangerous to the test community and the general public. We desire a certain level of realism in our operational tests, but we do not want to harm testers, soldiers, the general public, or the environment. Hence, we use the chemical and biological defensive system T&E paradigm (Holman 2002). That is to say, for each system under test we find a relatively harmless substance to

simulate each actual agent. That simulated agent has similar properties to the actual agent. Operational tests, field tests, and other tests with soldiers use relatively harmless simulated agents. Laboratory tests, in controlled chambers, use both actual agent and simulated agent. Based on laboratory tests, a transformation with correlation is made between the simulated agent and the actual agent. Using this transformation, we can then determine how the system under test would have performed with actual agent in an operational environment (Holman 2002).

Simulant

Simulants are relatively harmless substances that can be released in the environment to stimulate a biological detector or other chemical and biological defense system. The simulant is a surrogate for the biological warfare agent. A "good" simulant is one with properties that match the agent and is not harmful to the environment or test participants. The most critical property for detectors is that the detector performance when challenged with the simulant matches the performance with agent.

The national chemical and biological test and evaluation strategies were developed by the Test Capabilities and Methodology Integrated Product Team (TECMIPT). The TECMIPT was chartered by the Multiservice Test and Evaluation Executive for Chemical and Biological Defense. The national chemical and biological test and evaluation strategies state that simulants will be used in operational and field testing of chemical and biological defense systems. The main thrust of simulant testing is to determine system performance in an operationally realistic environment when the system is operated by representative users. Hence, there must be a strong relationship

between how the system under test responds to simulant, and how the system under test responds to agent (Radel, R 2005b).

The simulant selection process is complex and slow. The selection process should be based on the technology of the defensive system under investigation, the physical and chemical properties of the simulant and agent, safety, the threat, cost, Food and Drug Administration (FDA) requirements, Environmental Protection Agency (EPA) requirements, state requirements, the ability to measure and quantify concentrations, the needs of various simulant users, and operational test and evaluation priorities (Mahle et al., 2005).

The agent simulant knowledge base is a tool to aid in the simulant selection process. It was developed by US Army Edgewood Chemical Biological Center. It contains data on 2,500 chemical materials, 560 bacteria, 60 viruses, and 250 toxins. The system is easy to use and data queries can be made on either materials or properties. Unfortunately, the knowledge base has several shortfalls. Only 22 percent of the database is approved for public release. Many fields in the knowledge base are empty and over half of the fields have data that can not be traced to the original source. Despite these shortfalls this knowledge base system is a powerful tool for working with simulants and agents (Jablonski and Ashman 2005).

ARTEMIS is a standoff chemical detector. The program has been terminated. Tracy et al., (2005) discusses the lessons learned from simulant selection for ARTEMIS and, based on those lessons learned, proposes the development of a new tool. Simulant selection for ARTEMIS was an elaborate, expansive, and resource consuming effort. The selection criteria included the physical and chemical properties of the simulant and

agent, safety, the threat, cost, Environmental Protection Agency (EPA) requirements, state requirements, the ability to measure and quantify concentration, and operational test and evaluation priorities. The test and evaluation integrated product team evaluated 3600 potential simulants to arrive at five simulants that were used in testing. Tracy et al., (2005) propose that computational chemistry procedures and molecular modeling offer a potentially useful tool that could to be used in simulant selection (Tracy et al., 2005). Their concept is to start with the molecular structure of the chemical warfare agent of interest and then using computational chemistry and molecular modeling software alter the functional group that causes toxicity and calculate the desired properties. They propose iterating through thousands of potential simulants to produce a rank ordered list of candidate simulants.

Battat (2005) focused on operational realism of simulant testing. The use of simulants should parallel the use of agents and incorporate threat realistic concepts of operation and the enemy's tactical and strategic intent. Validation and accreditation of the simulant should not be limited to the physical characteristics of the agent and simulant, but rather should include a determination that the procedures in the test portray threat realistic concepts of operation and the enemy's tactical and strategic intent.

In agreement with Battat (2005), Touchton (2005) articulates that validation and accreditation of the simulant should not be limited to the physical characteristics of the agent and simulant, but must include a determination that the procedures in the test properly portray a realistic threat.

Hanley and Foarde (2005) focused on nuclear, biological, and chemical protective garment testing using aerosol simulants. They pioneered this methodology. The aerosol

simulant is used to test protection from nuclear particles and aerosolized biological warfare agents. This test has been used in the test and evaluation of the complete family of Joint Service Lightweight Integrated Suit Technology (JSLIST).

Clementi and Stevens (2005) used their experience with individual protection equipment simulants to suggest that the test and evaluation community should capitalize on simulants that were used by the science and technology community during system development. Simulants used during system development have been previously characterized. Use of these simulants during test and evaluation could save both time and money.

Unfortunately, many of the simulants used in system development are intended only to be used in a laboratory and hence often do not meet the safety and environmental constraints to be released into the environment.

Simulants that have been used to test decontamination solution have historically not reacted with the decontamination solution in a manner that even remotely represents how the decontaminant reacts with actual agent. As a result of this observation Shepherd (2005) is working on developing reactive decontaminant simulants. The mechanism by which a reactive decontaminant simulant reacts with the decontaminant should be the same as the reaction between the chemical warfare agent and the decontaminant. Furthermore the kinetics on the reaction between the reactive decontaminant simulants and the decontaminant should be similar to the kinetics on the reaction between chemical warfare agents and the decontaminant. To complicate the process of finding acceptable reactive decontaminant simulants, none of the products produced in the reaction between

reactive decontaminant simulants and decontaminant can be toxic or harmful to the environment.

Tevault (2005) discussed the simulant research program at Edgewood Chemical and Biological Center. In addition to the traditional threats of chemical warfare agents and biological warfare agents, they have started a new program on finding simulants for Toxic Industrial Chemical (TIC) Detectors and Toxic Industrial Material (TIM) Detectors. Many diverse TICs and TIMs are stored in industry and transported by truck and rail. Releasing TICs and TIMs from the industrial and transportation infrastructure is a convenient method for terrorists and enemies to launch a chemical attack. Another interesting program at Edgewood Chemical and Biological Center is directed at physical and chemical changes that occur in simulant and agents after release into the environment. It is well known that biological and chemical warfare agents undergo both physical and chemical changes after release. Chemicals in the atmosphere and physical parameters such as ultraviolet light alter both chemical and biological warfare agents after release. Hence, a simulant which matches most of the properties of an agent prior to release may in fact match few properties after the release. They propose that the simulant selection process should include a comparison of properties after the release of the simulant and agent.

As we have just seen, the environment may alter the properties of both simulants and agents. Palya (2005) points out different environments may alter both agents and simulants in different ways. For instance, if a simulant is altered by ultraviolet light, a release in a bright sunny environment will alter the simulant far more than a release at night.

If the simulant undergoes chemical reactions during release and use, then the simulant selection process must consider these reactions and becomes more complex (Tevault 2005). Simulants are used in testing biological warfare detection systems, chemical warfare agent detection systems, Toxic Industrial Chemicals (TICs) Detectors, Toxic Industrial Material (TIMs) Detectors, medical systems, decontamination systems, individual protection equipment, and collective protection equipment.

Radel (2005a) describes the simulant programs at Dugway Proving Ground (DPG). All of the biological warfare agent simulants which are used in testing were developed at DPG. Many of the chemical warfare agent simulants were also developed at DPG. DPG uses simulants to test biological detectors, chemical detectors, protective garments and masks, decontamination systems, collective protection systems, and medical diagnostic systems. DPG has a number of simulant testing facilities such as the Aerosol Simulant Exposure Chamber (ASEC), the Ambient Breeze Tunnel (ABT), the Standoff Ambient Breeze Tunnel (SABT), the Man In Simulant Test (MIST) chamber, and the decontamination pad. The ASEC is a 5 meters by 5 meters by 3 meters stainless steel Bio-Level 1 (BL-1) facility. It is used for testing systems with BL-1 simulants. The ABT is a BL-1 wind tunnel. It is 46 meters long, 6 meters wide and 6 meters tall. It is used for testing systems with BL-1 simulants. Both the ASEC and ABT are described in greater detail in chapter 3. The SABT is much larger than the ABT and is used to test standoff detectors. The MIST is used to test protective garments and masks against a vapor threat. In addition, simulants are often released in open air on various test ranges.

On 8 May 2007, in support of their simulant research and development mission, the Defense Threat Reduction Agency held a simulant summit. That summit produced

two reports, one for biological simulants (DTRA 2007a) and one for chemical simulants (DTRA 2007b).

The biological simulant summit report concluded that prior to simulant selection the scope of the system of interest must be defined, prior to simulant development one should determine if existing simulants are adequate, a simulant accreditation process should be developed, new sources for simulant development are needed, and criteria for simulant selection were proposed. The scope of the system of interest includes the intended application. Simulants used for calibration, training, evaluating the man-machine interface, testing one system, or testing a group of systems will have different requirements and constraints. One simulant that is acceptable for one system or application may not be useful to a different system or application. Documentation which could be used to determine the adequacy of existing simulants for the new application include the ASK program and NCBI PubMed. The ASK program can be used to perform database operations and analysis to facilitate comparing different simulants and agents. NCBI PubMed provides a search engine to query biological and medical publications. The summit recommended formalizing the simulant accreditation process. They suggested that the process should be similar to the FDA certification or ISO-9000 process. They proposed that new sources of simulant developers should be sought out. This could be accomplished publishing notice in scientific and engineering journals and also in trade publications. They conclude by recommending that the simulant selection process should include safety, traceability, classification, threat realism, operational relevance, simulant survivability, shelf life, regulatory issues, quality assurance, quality control, cost, and producibility (DTRA 2007a).

The chemical simulant summit report focused on the simulant selection process which is applicable for both chemical and biological simulants. The simulant selection process that they examined is a six step process. The first step is to define test requirements. The conditions to be replicated in the test and what are the peculiarities of the system under test are articulated and documented during this step. The second step is to define how the system under test interacts with actual agent. This step draws a picture of how the system works. Physical parameters such as temperature and humidity that effect how the system interacts with agent are enumerated and defined. The physical parameters are rank ordered in terms of importance. The third step is to define the desired simulant characteristics. The desired characteristics are determined based on the physical parameters identified in step two and the test requirements from step one. Cost, safety, and availability may also be desired characteristics identified during this step. Step four is to identify candidate simulants. Based on the required characteristics, candidate simulants are identified. Step five is to assess the performance of the candidate simulant. The assessment is based on quantitative acceptance criteria, quantitative agent-system under test performance, quantitative simulant-system under test performance, and analysis of the agent-simulant relative performance against acceptance criteria. This step leads to validation and verification. The last step is accreditation based on the validation and verification (DTRA 2007b).

Biological Simulants

Bacillus globigii (BG) also known as *Bacillus subtilis* is a spore forming soil bacterium. It has been used as a simulant for *Bacillus anthracis* with immunoassay technology (McBride et al., 2003). BG has been extensively used by the Army and Air

Force to simulate *Bacillus anthracis* for many detector technologies such as particle counters, immunoassay, florescent particle counters, mass spectrometry, and flow cytometers (Chipman et al., 2001, Christian and Holman 1995, Farrow 2002, Holman et al., 2004, Holman and Sleeper 1996a, Holman and Sleeper 1996b, Krozga and Holman 1995, Krozga and Holman 1996, Musgrave and Holman 1997, Musgrave et al., 2000, Peck 2002, Radel 2005a, Thermos et al., 2000, Thermos et al., 2001, Thermos et al., 2002a, Thermos et al., 2002b). At Dugway Proving Ground and Eglin Air Force Base BG has been released into the environment from aircraft, moving trucks, man portable backpack sprayers, and stationary devices and is reported in the following (Chipman et al., 2001, Christian and Holman 1995, Farrow 2002, Holman et al., 2004, Holman and Sleeper 1996a, Holman and Sleeper 1996b, Krozga and Holman 1995, Krozga and Holman 1996, Musgrave and Holman 1997, Musgrave et al., 2000, Peck 2002, Radel 2005a, Thermos et al., 2000, Thermos et al., 2001, Thermos et al., 2002a, Thermos et al., 2002b). There is substantial genetic variability among different strains of BG (Hofacre and Burke 2005). Hence, results from testing using one strain may not be representative of the whole species (Hofacre and Burke 2005).

Bacillus Thuringiensis (BT) is a spore forming bacterium. It has been used as a simulant for *Bacillus anthracis* with Polymerase Chain Reaction (PCR) and micro array technology (Song et al., 2006).

Erwinia herbicola (EH) is a vegetative bacterium. It has been used as a simulant to represent vegetative bacterial biological warfare agents such as *Yersinia pestis*, *Francisella tularensis*, *Brucella suis*, *Brucella melitensis*, *Brucella abortus*, *Brucella canis*, *Burkholderia mallei* (formerly known as *Pseudomonas mallei*) and *Burkholderia*

pseudomallei for immunoassay technology (McBride et al., 2003). EH has been used extensively by the Army and Air Force to simulate threat vegetative bacteria for other detector technologies such as particle counters, immunoassay, florescent particle counters, mass spectrometry, and flow cytometers and is reported in the following (Chipman et al., 2001, Christian and Holman 1995, Farrow 2002, Holman et al., 2004, Holman and Sleeper 1996a, Holman and Sleeper 1996b, Krozga and Holman 1995, Krozga and Holman 1996, Musgrave and Holman 1997, Musgrave et al., 2000, Peck 2002, Radel 2005a, Thermos et al., 2000, Thermos et al., 2001, Thermos et al., 2002a, Thermos et al., 2002b). At DPG EH has been released into the environment from moving trucks, man portable backpack sprayers, and stationary devices (Chipman et al., 2001, Christian and Holman 1995, Farrow 2002, Holman et al., 2004, Holman and Sleeper 1996a, Holman and Sleeper 1996b, Krozga and Holman 1995, Krozga and Holman 1996, Musgrave and Holman 1997, Musgrave et al., 2000, Peck 2002, Radel 2005a, Thermos et al., 2000, Thermos et al., 2001, Thermos et al., 2002a, Thermos et al., 2002b).

Male Stereotype 2 (MS2) is a bacteriophage. A bacteriophage is a virus that infects bacteria. It has been used as a simulant for biological warfare viruses such as smallpox and hemorrhagic fever viruses with immunoassay technology (Thomas et al., 2004, McBride et al., 2003). MS2 has been extensively used by the Army and Air Force to simulate threat viruses for detector technologies such as particle counters, immunoassay, florescent particle counters, mass spectrometry, and flow cytometers and is reported in the following (Chipman et al., 2001, Christian and Holman 1995, Farrow 2002, Holman et al., 2004, Holman and Sleeper 1996a, Holman and Sleeper 1996b, Krozga and Holman 1995, Krozga and Holman 1996, Musgrave and Holman 1997,

Musgrave et al., 2000, Peck 2002, Radel 2005a, Thermos et al., 2000, Thermos et al., 2001, Thermos et al., 2002a, Thermos et al., 2002b). At DPG MS2 has been released into the environment from moving trucks, man portable backpack sprayers, and stationary devises (Chipman et al., 2001, Christian and Holman 1995, Farrow 2002, Holman et al., 2004, Holman and Sleeper 1996a, Holman and Sleeper 1996b, Krozga and Holman 1995, Krozga and Holman 1996, Musgrave and Holman 1997, Musgrave et al., 2000, Peck 2002, Radel 2005a, Thermos et al., 2000, Thermos et al., 2001, Thermos et al., 2002a, Thermos et al., 2002b).

Ovalbumin (OV) is extracted from egg whites. It is a protean mixture. It has been used as a simulant for toxins for immunoassay technology (McBride et al., 2003). OV has been extensively used by the Army to simulate threat toxins for detector technologies such as particle counters, immunoassay, florescent particle counters, mass spectrometry, and flow cytometers and is reported in the following (Chipman et al., 2001, Christian and Holman 1995, Farrow 2002, Holman et al., 2004, Holman and Sleeper 1996a, Holman and Sleeper 1996b, Krozga and Holman 1995, Krozga and Holman 1996, Musgrave and Holman 1997, Musgrave et al., 2000, Peck 2002, Radel 2005a, Thermos et al., 2000, Thermos et al., 2001, Thermos et al., 2002a, Thermos et al., 2002b). At DPG OV has been released into the environment from moving trucks, man portable backpack sprayers, and stationary devises (Chipman et al., 2001, Christian and Holman 1995, Farrow 2002, Holman et al., 2004, Holman and Sleeper 1996a, Holman and Sleeper 1996b, Krozga and Holman 1995, Krozga and Holman 1996, Musgrave and Holman 1997, Musgrave et al., 2000, Peck 2002, Radel 2005a, Thermos et al., 2000, Thermos et al., 2001, Thermos et al., 2002a, Thermos et al., 2002b).

Killed agent like organisms (ALO) and inactivated toxins/toxoids are relatively new simulants. ALO include vaccine strains and non-pathogenic bacteria and viruses that are phylogenetically closely related to the biological warfare agent (Fitch et al., 2004, Radel 2005a). At DPG, ALOs have been released into the environment from stationary spray devices (Radel 2005a).

"Bugbeads" are polystyrene beads coated with the outer surface of a bacteria. The outer surface could be cell membrane or spore coat. Bugbeads have been used to simulate BG and could be used for other pathogenic bacterial agents (Farrell et al., 2005, Hofacre and Burke 2005).

National Research Council Review of T&E of Biological Point Detectors

The National Research Council of the National Academies undertook a review of the Department of Defense test and evaluation methodology for biological point detector systems (Fitch et al., 2004). This section provides an overview of their findings.

The review was initiated by the Department of Defense Joint Program Executive Office for Chemical and Biological Defense for the purpose of improving the test and evaluation of biological point detector systems.

Of the four most commonly used simulants -- *Bacillus subtilis, Erwinia herbicola*, Male Specific (MS2) bacteriophage, and Ovalbumin, the committee found that only *Bacillus subtilis* is a reasonable surrogate for a full range of biothreat agent properties. The properties that they considered were stability to aerosolization, transport properties, surface hydrophobicity and hydrophilicity, surface morphology and stability, antigenicity, and genomic stability and similarity. They also concluded that new simulants are needed and recommended the use of killed ALOs such as the vaccine strain

of anthrax. They also said that there should be at least one simulant per type of biological warfare agent. The better the simulant copies or mimics the biological warfare agent properties, the more confidence that we will have that the detector is performing with the simulant in the same manner as it would with that specific biological warfare agent. An ideal simulant will have the following properties:

- interact with the system under test in a manner that can be directly correlated and related to a biological warfare agent,
- be able to be presented to the system under test in a threat realistic manner,
- concentration and other attributes can be quantified,
- be safe to use.

The committee also recommended that a whole system live agent test facility be constructed and that analytical methods be developed and used to relate detector performance with live agent in the chamber to field performance. This recommendation from the National Research Council contributed greatly to the selection of this dissertation topic. This dissertation is the first effort to implement their recommendations.

Agent-Simulant Transformations

Despite the fact that much is known about agent and simulant properties, surprisingly little has been accomplished on agent simulant correlations (Kaufman 2005).

Early evaluations of biological detectors either assumed that the detector performance against agent was equivalent to performance against simulant; or used performance against 2 or more simulants to bound the performance against agent (Holman 2002).

The US Army Test and Evaluation Command has four separate and different efforts to develop agent-simulant transformation methodologies. Each of these efforts is employing a different methodology. These are the only efforts to develop the agent-simulant transformation. These four efforts will support the Joint Service Lightweight Standoff Chemical Agent Detector (JSLSCAD), Joint Chemical Agent Detector (JCAD), Joint Biological Standoff Detection System (JBSDS), and the Joint Biological Point Detection System (JBPDS). The rationale in developing four separate methods is threefold:

1. When the simulant development program was initiated, the detector program was well underway. The individual programs had unique attributes that favored a specific agent-simulant transformation.
2. It is not clear which agent-simulant transformation method would be successful.
3. There will be an opportunity to compare and contrast the different agent-simulant transformation methodologies. Lessons learned will provide an opportunity to develop some basic principles for developing agent-simulant transformation methods.

JSLSCAD Agent-Simulant Transformations

JSLSCAD is a stand off chemical warfare detector. It will be mounted in reconnaissance vehicles and provide information on the chemical threat in both the surveillance and reconnaissance missions.

The JSLSCAD agent simulant transformation and results are described in Holman et al., 2007a, Holman et al., 2007b, Holman et al., 2006a Holman et al., 2006b, Holman

and Winslow 2006 and Holman et al 2008. The methodology uses the following hierarchy of models:

- Vapor, Liquid, Solid Tracking model (VLSTRACK) is used to develop a three-dimensional cloud with concentration over time. The cloud development depends on the threat agent, delivery method, meteorological conditions, and the terrain.
- Chemical Biological Analyzer (CB Analyzer) converted VLSTRACK model results to Concentration length (CL) based upon the temporal and spatial relationship between the JSLSCAD and the cloud. CL is the product of the concentration and the length of the vapor cloud on the axis that JSLSCAD is sensing the cloud.
- The JSLSCAD model provides JSLSCAD performance for a non scanning system based upon the CL, ΔT, range to cloud, and spectra. ΔT is the difference between the temperature of the vapor cloud and the background.
- The scan model links the three models together and represents a single sensor interrogating the same cloud multiple times. With the CB Analyzer, it was used to relate the scan pattern of the JSLSCAD to the threat cloud.
- ΔT and background spectra data were collected at specific times of the day at tactical locations of interest and were input to the M&S.

- Agent spectral data were provided by Pacific Northwest National Laboratory (PNNL) and WDTC.

In addition, based on spectral signatures of simulant, chemical warfare agent, and the JSLSCAD model, simulant concentrations were adjusted during field tests so that from the prospective of JSLSCAD the brightness of the simulant spectral signature was equivalent to the brightness of chemical warfare agent.

JBSDS Agent-Simulant Transformations

JBSDS is a standoff biological warfare agent detector. It can be mounted on a vehicle or installed at a fixed site location. It provides information on biological warfare agents during a surveillance mission.

The JBSDS agent-simulant transformation uses the JBSDS software, precise laboratory measurements on the florescent cross sections and backscatter of agents and simulants, and the system log from field tests. The system log records all of the input data to the system algorithm from the simulant release. The agent simulant transformation for JBSDS is based on changing the input signal from simulant to agent in the system log and re-running the signal through the software with the same background noises as were seen in the field with the simulant. This will produce performance results that are equivalent to what would have been expected if actual biological warfare agent had been released during the test. The method has been verified by changing the florescent cross sections and backscatter of one simulant to a different simulant and comparing the predicted results to actual test results (Shirakawa et al 2008).

JCAD Agent-Simulant Transformations

JCAD is a portable point chemical warfare agent detector that will be carried by warfighters and mounted on and in vehicles. It will provide information on chemical warfare agents in all missions.

The JCAD agent simulant transformation was to be based on classic multiple logistic regression. Unfortunately, the instrumentation used in the operational test to quantify concentration was not sensitive enough to support this analysis. An adequate agent-simulant transformation could not be developed (Holman et al., 2007).

JBPDS Agent-Simulant Transformations

JBPDS is a point biological detection system. Since it can not operate on the move, it will be used in surveillance missions. It can, however, be employed in "bounding over watch". A more detailed description of JBPDS is found in chapter one.

The JBPDS agent simulant transformation is based on a heuristic logistic regression developed by Holman, Russell, and Jennings (2004). In this procedure, logistic regression models for detector performance for both biological warfare agent and simulant based on laboratory results are developed. A logistic regression model is also developed for detector performance based on simulant in the field. The aim is to develop a model to predict the probability of detection based on the concentration of agent or simulant in the air. Other factors such as particle size may be considered in this model. A heuristic logistic regression model to predict the probability of detection is developed by using the simulant field shape parameter β and the laboratory agent shift parameter α. A comparison of the laboratory and field shift parameter α is used to determine if additional adjustment is needed for the agent shift parameter. The basic theory which

supports this method is that aerosol concentration in the lab is constant and easy to measure while aerosol concentration in the field is more variable and difficult to measure at the exact location of the biological agent detector. Hence, detection models based on laboratory data produce shape parameter with steeper 's' curves than if the model is based on field data.

This heuristic logistic regression is one of the focuses of this dissertation.

Logistic Regression

An overview of logistic regression can be found in Agresti (1996). A more detailed discussion can be found in Hosmer and Lemeshow (1989) and Allison (1999). This section is based on those three references and provides the bases for both the JCAD agent-simulant transformations and the JBPDS agent-simulant transformations.

Logistic Regression is a special case of generalized linear models. General linear models are a broad class of models that includes ordinary regression and analysis of variance (ANOVA). All general linear models consist of three parts. They are the random component, the systematic component, and the link.

- The random component identifies the response variable Y. It assumes a probability distribution for Y. Y_is are treated as independent.
- The systematic component specifies the explanatory variables used in the model to predict the response Y. The systematic component can be expressed as a linear combination of explanatory variables as follows:

$$\alpha + \beta_1 x_1 + \ldots + \beta_k x_k$$

However, some x_is may be based on non-linear models. For instance one of the x_is could be equal to x raised to a power.

- The link between the random component and the systematic component specifies how the expected value of the response variable E(Y) relates to explanatory variables in the linear component. The link is expressed as:

$$\mu = E(Y)$$

The simplest link function is simply E(Y) is equal to the mean of an explanatory variable. The link for ordinary regression is:

$$\mu = \alpha + \beta_1 x_1 + \ldots + \beta_k x_k$$

The link function may be linear or nonlinear.

Categorical response variables often have only two categories. Examples of two category response variables include political party – republican versus democrat, Flip a coin – head versus tail, bet – win versus lose, and biological detection system – detect versus don't detect. These binary responses of Y are often called Bernoulli variables and are typically denoted by success and failure or by 1 and 0. This distribution has a mean $E(Y) = \pi$ and a variance $Var(Y) = \pi(1 - \pi)$. For n binary independent experiments or observations with parameter π the number of successes has a binomial distribution specified by n and π.

The logistic regression model is one of several general linear models which can address response variables with only two categories. In the logistic regression model:

- Random component is categorical and typically binary.

- The systematic component specifies the explanatory variables used in the model to predict the response Y.
- The link between the random component and the systematic component is a sigmoid shaped function known as logit link. The logit link produces $0 \leq \mu \leq 1$ and is of the form:

$$\mu = \log(\mu / (1- \mu))$$

The logistic regression function can also be expressed as:

$$\text{Log}(\pi(x)/1 – \pi(x)) = \alpha + \beta x$$

This will result in:

$$\pi(x) = \exp(\alpha + \beta x) / 1 + \exp(\alpha + \beta x)$$

The logistic regression model is a sigmoid shaped function in which a fixed change in x produces a smaller change in π when π is close to 0 or 1 than it does when π is in the middle of the range (see figure 2-1). In this equation α is a shift parameter. When α equals zero, the curve steepest slope is at x equal zero and the probability of x equal zero is 0.5. As the shift parameter α becomes more positive the curve shifts to the left (see figure 2-2). As the shift parameter α becomes more negative the curve shifts to the right (see figure 2-2). $\beta = 0$ results in $\pi(x)$ being a flat horizontal line. $\beta > 0$ yields a monotonically increasing function for $\pi(x)$. $\beta < 0$ yields a monotonically decreasing function for $\pi(x)$. As the shape parameter β becomes larger in absolute value, the slope becomes steeper (see figure 2-3). As the shape parameter β becomes smaller in absolute value, the slope becomes less steep (see figure 2-3).

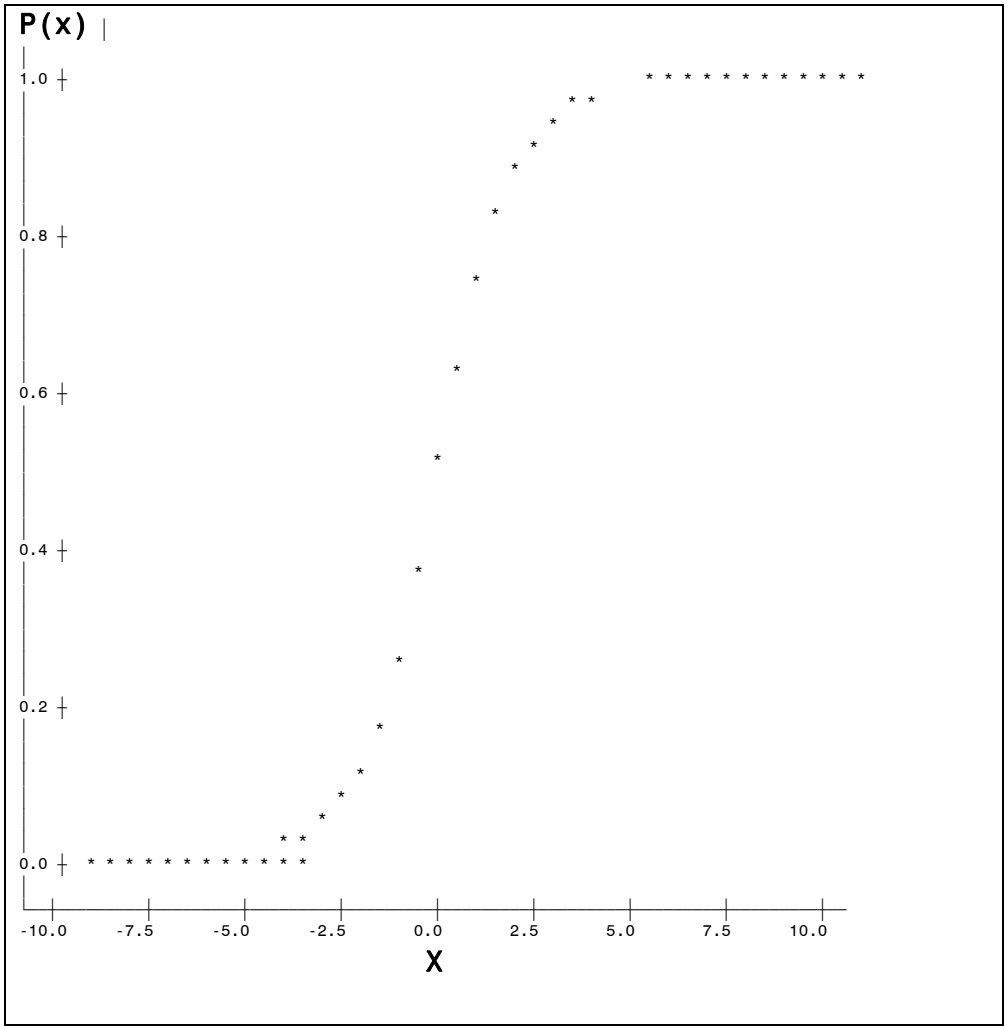


Figure 2-1 Plot of the probability of X (P(X)) versus X for the logistic regression curve: P(X)=exp(0+1x)/(1+exp(0+1x))

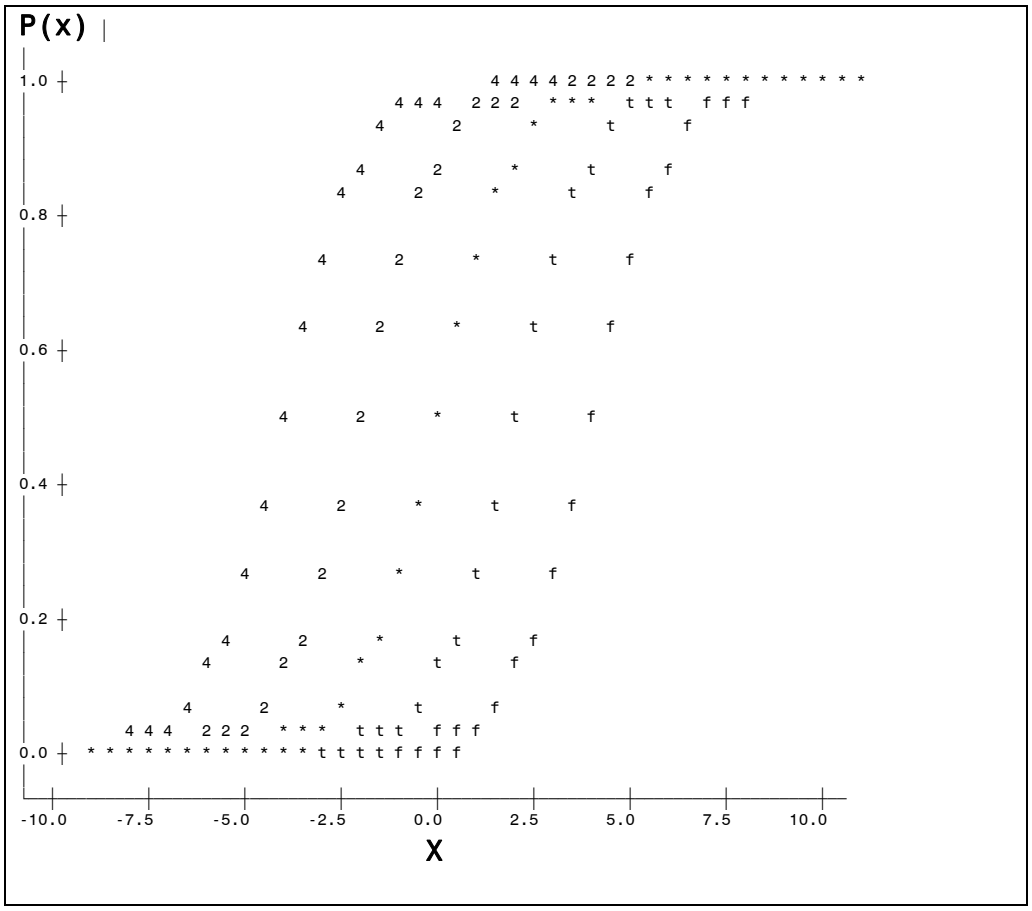


Figure 2-2 Plot of the probability of X (P(X)) versus X for the logistic regression curve: $P(X)=\exp(\alpha + \beta x)/(1+\exp(\alpha + \beta x))$. This series of plots depicts the effect of changing the shift parameter α while holding β constant. As the shift parameter α becomes more positive the curve shifts to the left. As the shift parameter α becomes more negative the curve shifts to the right.

Note: P(X)=exp(4 + x)/(1+exp(4 + x)) is plotted with the symbol '4'
P(X)=exp(2 + x)/(1+exp(2 + x)) is plotted with the symbol '2'
P(X)=exp(0 + x)/(1+exp(0 + x)) is plotted with the symbol '*'
P(X)=exp(-2 + x)/(1+exp(-2 + x)) is plotted with the symbol 't'
P(X)=exp(-4 + x)/(1+exp(-4 + x)) is plotted with the symbol 'f'

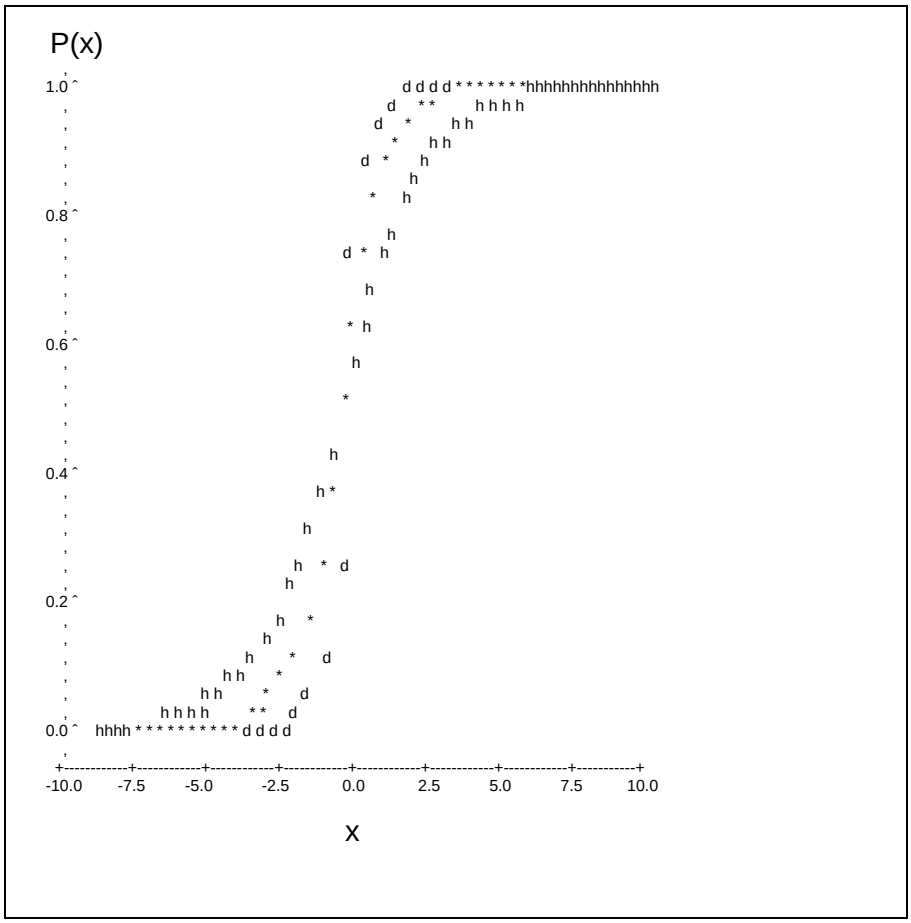


Figure 2-3 Plot of the probability of X (P(X)) versus X for the logistic regression curve: $P(X)=\exp(\alpha + \beta x)/(1+\exp(\alpha + \beta x))$. This series of plots depicts the effect of holding the shift parameter α constant while changing the shape parameter β. As the shape parameter β becomes larger in absolute value, the slope becomes steeper. As the shape parameter β becomes smaller in absolute value, the slope becomes less steep.

Note: $P(X)=\exp(0 + 2x)/(1+\exp(0 + 2x))$ is plotted with the symbol 'd'
$P(X)=\exp(0 + x)/(1+\exp(0 + x))$ is plotted with the symbol '*'
$P(X)=\exp(0 + 0.5x)/(1+\exp(0 + 0.5x))$ is plotted with the symbol 'h'

Current DoD Basic Research on Simulants

The Defense Threat Reduction Agency, Joint Science Technology Office is charged with execution of the Department of Defense simulant basic research. Currently they have four simulant basic research efforts. They are developing a simulant selection process, developing simulants to be used on protective equipment, developing polymer microsphere technology as simulants, and developing prion, mustard, and poxvirus simulants for use with decontamination systems (Kaufman 2007).

The development and demonstration of simulant selection process will focus on simulants that represent chemical warfare agents during a decontamination process. The process will be represented as computer software. It will employ empirical and theoretical data to model the CWA decontamination process. Other models on molecular reactions and properties of similar compounds will be used to identify potential simulants (Kaufman 2007).

The development of simulants to be used on protective equipment will include both individual protective equipment and collective protective equipment. It will focus on simulants for the following chemical warfare agents: VX, soman (GD), distilled mustard (HD), and Nontraditional Agents (NTA) (Kaufman 2007).

The development of polymer microsphere technology as simulant of microorganisms will focus on replicating the fluorescence signature of biological warfare agents on the polymer microspheres. In this effort fluorophores will be combined in appropriate amounts and ratios to produce the desired fluorescence signature of a biological warfare agent. Fluorophores are florescent dyes that are typically used in

research. Fluorophores molecules have a functional group that will absorb energy in a specific wavelength and re-emit that energy in a different wave length (Kaufman 2007). The development of decontamination simulants will focus on simulants for prions, mustard, and smallpox. This effort will examine several decontamination fluids. A yeast prion will be used as a simulant for infectious human prions. Yeast prions are substances that, from a molecular standpoint, are similar to the prion that causes mad cow disease. Methyl salicylate (MeS) will be used as a simulant for mustard. A non-human infectious pox virus will be used to simulate smallpox. The efficacy of the decontamination solution to the simulants will be compared to the efficacy of the of the decontamination solution to the agent (Kaufman 2007).

Chapter 3 Methods

This chapter will address both testing and analytical methods.

The data for this dissertation were developed at Dugway Proving Ground (DPG) under the Whole System Live Agent Test (WSLAT) methodology project and the Army portion of the JBPDS Multiservice Operational Test with ALOs. The purpose of WSLAT is to provide an environment and associated methodologies to facilitate the evaluation of JBPDS. WSLAT provides data to evaluate JBPDS detection and presumptive identification against one live and one killed biological simulant; four live and four killed BWA representing spore-forming bacteria, vegetative bacteria, viruses and toxins; and four live and four killed ALO aerosols (that correspond to the four BWA). The overall process will extrapolate all chamber testing to the field. A more detailed description of WSLAT data can be found Holman et al. (2006c).

This chapter will provide an overview of the different chambers used in the WSLAT, the release matrix of agents and simulants, information on instrumentation, the definitions of principal measures, and the hypotheses to be tested.

CAC Chamber Testing

The live agent Containment Aerosol Chamber (CAC) is the only Bio-Level 3 (BL-3) facility in the WSLAT process. Hence, the CAC environment provides the only opportunity to determine the performance of JBPDS against live biological warfare

agents. The CAC is a stainless steel structure with glass windows, glove ports, and a half-suit. Objects in the CAC can be manipulated by the glove ports or the half-suit. The CAC has a volume of 7 cubic meters. Agent and simulant disseminations occur in a small inner chamber which has a one cubic meter volume. Dissemination is accomplished by a Sono-Tek ultrasonic spray nozzle system. Referee systems and the JBPDS sample this small interior camber. All air entering or leaving the CAC is filtered by High Efficiency Particulate Air (HEPA) filters. Data on the JBPDS performance against the live BWA and performance against equivalent aerosol concentrations of the ALO and simulants will be made in the CAC. Because of chamber size constraints, the JBPDS components will not be assembled as if they were in an actual system, but rather they will be "strung-out" to accommodate the CAC chamber.

ASEC Chamber Testing

The Aerosol Simulant Exposure Chamber (ASEC) is used as a BL-1 facility. It is a stainless steel chamber. Like the CAC, all air entering or leaving the ASEC is HEPA filtered. It is 5 meters by 5 meters by 3 meters. Referee systems and the JBPDS were located inside the ASEC. Testing in the ASEC examined dissemination, air mixing, and aerosol measurement techniques for killed ALO, and both live and killed BG simulant. The output of this approach provided data to describe the probability of detection represented as P(D) and the resulting probability of identification represented as P(ID) of the JBPDS system for a given aerosol concentration for each killed ALO, and the BG simulant.

Ambient Breeze Tunnel (ABT) Testing

The ABT is a BL-1 facility. Environmental, non-filtered air enters the ABT. The ABT is 46 meters long, 6 meters wide and 6 meters tall. The top forms an arc. ABT testing provided data to evaluation of JBPDS detection and presumptive identification performance against the live and killed biological simulant and four killed ALO aerosols in a semi-contained chamber under ambient field conditions. This testing allowed a comparison of JBPDS detection and identification performance against a live simulant and killed simulant and killed ALO.

Field Testing

Field testing occurred as part of the Army portion of the multi service operational test. Field testing provided the final venue to evaluate detection and presumptive identification performance of the JBPDS against one live and killed biological simulant and four representative killed ALO in a field environment. The focus of JBPDS testing in the field is to investigate JBPDS performance against aerosols in a threat realistic environment. The output of this approach is to describe the P(D) and the resulting P(ID) of the JBPDS system for a given aerosol concentration for each ALO, and simulant.

WSLAT Methodology Developmental Test

WSLAT methodology trials started on 28 September 2006. The testing and data authentication were completed on 12 December 2007. This testing characterized the aerosols of four BWA along with the corresponding ALO and the simulant BG in the CAC, ASEC, and ABT. The characterization process includes (1) standardized agent preparation procedures consistent with the threat, (2) characterization of aerosols in each chamber environment in terms of concentration, particle size, and cloud profile, and (3)

certification of referee instrumentation used to measure aerosol characteristics, both directly and indirectly. This testing also characterized the JBPDS detection and identification performances when challenged with these aerosols of four BWA along with the corresponding ALO and the simulant BG in the CAC, ASEC, and ABT.

WSLAT methodology trials resulted in:

- 473 system challenges in the CAC
- 526 system challenges in the ASEC
- 1,214 system challenges in the ABT

The agent and simulant release matrix is depicted in table 3-1. All agents and simulants in this table were released in the CAC. The live and killed agents LE, NU, N, and XR and their corresponding live ALO were only released in the CAC and are in blue shaded cells of table 3-1. The live simulant BG and killed BG, killed LE ALO, killed NU ALO, killed N ALO, and deactivated XR were released in both the ASEC and ABT.

Test data was recorded and managed by DPG. Data authentication was accomplished by the WSLAT data authentication group and chaired by the author of this dissertation.

Table 3-1 Release Matrix of agents and simulants. All agents and simulants depicted in this table were released in the CAC. Simulants, to include killed ALOs that are not in shaded cells were released in the ASEC, ABT, and the field. Agents and simulants are: BG-*Bacillus subtilis, formerly Bacillus globigii*, LE-agent code, NU-agent code, N-agent code, XR-agent code. ALO is Agent-Like Organism

Live or Active Agent	**Killed Agent**	**Live Simulant**	**Inactivated Simulant**
		BG	Killed BG
LE	Killed LE	LE ALO	Killed LE ALO
NU	Killed NU	NU ALO	Killed NU ALO
N	Killed N	N ALO	Killed N ALO
XR			Deactivated XR

Test instrumentation

Slit sampler, Anderson cascade impactor, all glass impinger, aerodynamic partial samplers, anemometer, C-FLAPS, and the XMX are the principal instrumentation used in this effort and are described in the following sections. Instrumentation used to quantify the concentration and duration of an aerosol cloud is typically referred to as referee. The concentration is represented in Agent Containing Particles per Liter of Air (ACPLA).

Slit Sampler Agar (STA) Sampler

The slit sampler has long been the standard biological field testing referee. A large Petri plate filled with agar rotates on a turntable that is timed for a specific rotation period. The Petri plate is protected by a cover that has one narrow slit in it. When the mobile ground sampling system is turned on, a vacuum draws an air sample through the

slit at a flow of 28.3 L/min, and the particles in the air impact onto the agar. As the plate rotates, the area of the Petri plate impacted below the slit also moves. This provides a "clock" of particles impacted onto the plate. The plates are incubated, and the viable particles will grow. After the incubation period (usually 14 to 24 hours), the plates are placed on a scanner to count bacterial colonies that can be separated into time-resolved sectors to provide a measurement of ACPLA versus time. The ACPLA is the field standard for referee of bacterial concentration. Limitations: results are not available until after incubation and counting, usually 48 to 72 hours. Only viable bacteria can be assayed this way; viruses, proteins, and bacteria that do not withstand impaction cannot be assayed with this method. STA are used with a sampling time of 2 minutes to more fully characterize a challenge. Dycor® software is used to rotate trial plates in sequence.

Andersen Cascade Impactor (ACI)

The ACI is a particle-fractionating viable microbial sampler that separates particles according to their size and impinges them onto an agar medium collection surface. Incubation of the agar plates allows colony formation and quantification of the biological particles. Air is drawn by vacuum into the sampler at 28.3 L/min (1 ft^3/min), and then successfully through six stages with progressively smaller orifices. The jet velocity of the airstream increases with each stage, effectively separating particles into six size ranges: 9.2 µm and above, 5.5 to 9.2 µm, 3.3 to 5.5 µm, 2.0 to 3.3 µm, 1.0 to 2.0 µm, and 0.3 to 1.0 µm.

All Glass Impinger (AGI)

Air is drawn by a vacuum into a tube and into a liquid. As the air enters the AGI, it encounters a bend in the glass sampler. The bend is shaped like the human trachea, so

that only the particles that would naturally impact in the human lung are drawn into the AGI. An AGI sample can be collected for various time intervals; the typical length of a sample is 2 minutes. AGIs are also typically run in sequence using Dycor® software. Typically, the liquid in the AGI, for biological field testing, is phosphate-buffered saline (PBS). After the sample is collected, it can be assayed with various laboratory techniques, depending on the test requirements. A limitation in the use of the AGI is the destruction of some of the sample due to the violent collection method. The time resolution is limited to the duration of AGI collection, and only one data point is given for each AGI sample. During the methodology trials, both antigen-antibody and genetics-based assays will be used to measure the concentration of biological material in collected samples.

Aerodynamic Particle Sizer (APS®)

The TSI, Inc. (Shoreview, Minnesota) APS® 3321 is a time-of-flight spectrometer that measures the velocity of particles in an accelerating air flow through a nozzle. In the instrument, particles are confined to the centerline of an accelerating flow by sheath air. They then pass through two broadly focused laser beams, scattering light. Side-scattered light is collected by an elliptical mirror that focuses the collected light onto a solid-state photodetector, which converts the light pulses to electrical pulses. By timing the interval between the peaks of the pulses, the velocity can be calculated for each individual particle. Velocity information is stored in 1024 time-of-flight bins. Using a polystyrene latex sphere calibration, which is stored in nonvolatile memory, the APS Model 3321 converts each time-of-flight measurement to an aerodynamic particle diameter. For convenience, this particle size is binned into 52 channels. The particle size range

spanned by the APS is from 0.5 to 20 µm in both aerodynamic size and light-scattering signal. Particles are also detected in the 0.3 to 0.5 µm range using light-scattering alone, and are binned together in one channel. The APS is also capable of storing correlated light-scattering-signal data and time-of-flight data. Limitations to the APS are the inclusion in the particle count of sub-micron particles from sources other than the disseminator as well as its inability to distinguish agent-containing particles from other particles, and its similar inability to distinguish particles containing dead/damaged agent from those containing viable agent.

Anemometer

The R.M. Young Company (Traverse City, Michigan) Model 81000V ultrasonic anemometer is a 3-axis wind sensor without moving parts. It is designed to provide fast response, high resolution, 3-dimensional wind measurement up to 40 m/s with a resolution of 0.1 m/s, and 360-degree directionality with a resolution of 0.1 degree.

C-FLAPS

The C-FLAPS is a florescent APS. The front end of the C-FLAPS employs a three stage XMX/2A (air-to-air) particle concentrator that collects aerosol particles ranging from 1-10 microns. The Model 3317 Fluorescence Aerosol Particle Sensor III (FLAPS III)™ System provides three real-time measurements of individual airborne particles. These correlated single particle measurements give the C-FLAPS exceptional discrimination and interference rejection for biological threat detection applications. Fluorescence and scattered-light signals are excited using a 405 nm laser diode that provides high reliability and stability. Fluorescence emissions are measured using two highly sensitive photomultiplier tubes. HEPA-filtered sheath airflow introduced around

the inlet air stream limits the need for optics cleaning and increases system availability. The C-FLAPS software module reads data from FLAPS, logs all particle and fluorescence data, and provides real time display of the particle size, numbers and fluorescence data.

XMX Single Sample Aerosol Collector

The XMX/2L-MIL is an aerosol separator, sample preparation, and high mass flow concentrator system, design to operate under harsh field conditions. This system was designed to process and collect large concentrations of aerosols in the respirable range (1 to 10 microns in diameter) in relatively short periods of time, i.e., when the cloud is over the XMX. This system collects high volumes of air, strips away the large dust particles and the very small micro debris and concentrates the aerosols of interest. The particles are then impinged into a liquid sample collection vial (centrifuge tube) containing 5 milliliters of prepared sterile water or phosphate buffered saline solution or onto a retrofit dry filter. Once the sample is collected depending on the sample type either the liquid centrifuge tube or the dry filter is sealed for subsequent lab analysis (immuno-assay, PCR, or culturing).

XMX-102 Multi-Stage Collector

The XMX-102 is a modification of Dycor Technologies existing high volume (530 liters of air per minute) single sample XMX aerosol collector. The XMX-102 modification changes the single XMX aerosol collection system into a multiple collection system allowing up to 102 programmable time-sequenced sealed and disposable samples

to be collected. The XMX-102 software module reads data from the XMX-102, time tags and logs all sample events and provides real time health and status data of from the collector.

Field Test

The field test occurred at Dugway Proving Ground as the Army portion of the JBPDS multiservice operational test. Testing of seven JBPDS systems occurred over a two month period. Each system had a crew of four that worked in shifts, such that 2 operators were always present during the mission. As depicted in table 3-2, the JBPDS was challenged with killed ALO, and BG simulant aerosols in an open-air field environment. Field releases of biological aerosols were both ground and aerial. Aerial releases were all dry simulant. Ground releases included both wet and dry simulant. There were 504 system challenges during the field test. As with chamber testing, ALOs and BG were prepared IAW accepted threat preparation procedures. Releases were conducted in random order based on the release matrix below. Detection, identification and sampling data was collected for all systems under test.

Table 3-2 Field Challenge Matrix. This table identifies the simulants that were released in the field test. It identifies whether the simulants were alive or killed, wet or dry, and if they were released from the ground or airplane.

Challenge Material	State	Condition	Release Mode
BG	Live	Dry	Ground
BG	Live	Dry	Aerial
BG	Live	Wet	Ground
BG	Killed	Dry	Ground
BG	Killed	Dry	Aerial
BG	Killed	Wet	Ground
ALO Spore Bacteria	Killed	Dry	Ground
ALO Spore Bacteria	Killed	Dry	Aerial
ALO Spore Bacteria	Killed	Wet	Ground
ALO Vegetative Bacteria	Killed	Wet	Ground
ALO Virus	Killed	Wet	Ground
ALO Toxin	Inactive	Dry	Ground
ALO Toxin	Inactive	Dry	Aerial
ALO Toxin	Inactive	Wet	Ground

Measures

The following measures were made during testing:

- Particle size distribution for each agent/ALO/simulant in each relevant environment (CAC/ASEC/ABT/Fld). [for 10% of releases]
- Initial concentration (cfu/pfu/ng per ml) of each biological material slurry at the time of dissemination.
- Feed rate of biological material through dissemination nozzles for each trial.
- Average concentration of each biological aerosol in 1-minute (direct) and 2-minute (indirect) increments expressed in ACPLA and biological units/liter air.
- Peak concentration of each biological aerosol expressed in ACPLA and biological units/liter of air.

- Aerosol concentration of biological material aerosols in no more than 2-minute increments for indirect collection (AGI) methods expressed in both ACPLA and biological units/liter of air.
- Aerosol concentration of biological material aerosols in no more than 10 second increments for direct collection (STA) methods expressed in ACPLA and biological units/liter air.
- Start and stop time of each aerosol generation.
- Aerosol concentration profiles over time in both ACPLA and biological units/liter of air for each trial.
- Temperature range (degrees C).
- Relative humidity.
- Air velocity within each WSLAT containment chamber (1-6 ± 0.1 meters/sec).
- Time agent, ALO, or simulant is first detected.
- Number of detection events that agent or simulated agent is identified correctly to species divided by the total number of valid detection events (probability of identification point estimate) in each environment.

Hypotheses

This dissertation addresses two issues relevant to the testing and evaluation of biological warfare detectors. The issues are:

1. How acceptable are killed ALOs as simulants for representing live biological warfare agents during biological warfare agent detector field tests?

2. How acceptable is simple modeling based on logistic regression method to predict biological warfare detector and identifier performance against live biological warfare agent?

Both of these issues will be analyzed and evaluated using results of experiments or tests conducted at Dugway Proving Ground. These data are described prior sections in this chapter. This analysis and evaluation will be based on JBPDS detection and identification performance in both field and chamber testing. JBPDS is described in chapter one. JBPDS detection uses fluorescence of particles. Most of the fluorescence is generated by the amino acid tryptophan. JBPDS identification is based on immunoassay strips. Both of these issues, the acceptability of killed ALOs as simulants and the acceptability of the models will be addressed in the context of both hypothesis testing and judgment.

These issues will be evaluated within the framework of 5 hypotheses. The remaining part of this chapter will provide a discussion of each hypothesis to be evaluated statistically. The hypotheses are as follows.

- Hypothesis 1: (live versus killed hypothesis)
  - Ho: JBPDS performance is the same with killed and live agent (or simulant).
  - Ha: JBPDS performance is different for killed and live agent (or simulant).
- Hypothesis 2: (live ALO versus live agent hypothesis)
  - Ho: JBPDS performance is the same with live agent or the corresponding live ALO.

  - Ha: JBPDS performance is not the same with live agent as it is with the corresponding live ALO.

- Hypothesis 3: (acceptable simulant hypothesis)
  - Ho: JBPDS performance is the same with killed ALO and live agent.
  - Ha: JBPDS performance with killed ALO is different from its performance with live agent.
- Hypothesis 4: (chamber effect hypothesis)
  - Ho: JBPDS performance is the same in all chambers (CAC, ASEC, ABT, and the field)
  - Ha: JBPDS performance is not the same in all chambers (CAC, ASEC, ABT, and the field.
- Hypothesis 5: (modeling hypothesis)
  - Ho: JBPDS performance can be predicted with a model based on logistic regression.
  - Ha: JBPDS performance cannot be predicted with a model based on logistic regression

Rejecting the third or forth hypotheses provides motivation to evaluate the fifth hypothesis. The first two hypotheses provide clarity on the mechanism leading to the conclusion of the third hypothesis.

Killed ALO as Simulants

Note Because JBPDS performance with agent is classified, biological warfare agent performance results will NOT be reported in the dissertation. Detailed results will be reported for BG. For all BG cases both cumulative probability vs concentration plots,

and logistic regression plots will be displayed. Only statistical p values and other statistical parameters such as R^2 which are not relatable to actual JBPDS performance will be reported for biological warfare agent.

Hypothesis 1:

- H_o: JBPDS performance is the same with either killed or live agent.
- H_a: JBPDS performance is different for killed and live agent.

CAC (BL3): Classical logistic regression statistical models will be constructed for the probability of detection as a function of concentration and for the probability of identification as a function of concentration for each of the following: 1. BG, 2. BWA Spore Bacteria crude, 3. BWA Spore Bacteria washed, 4. BWA Spore Bacteria washed twice, 5. ALO Spore Bacteria crude, 6. ALO Spore Bacteria washed twice, 7. BWA Vegetative Bacteria, 8. ALO Vegetative Bacteria , 9. BWA Virus, and 10. ALO virus. For each of the 10 cases a statistical test will be performed for both detection and identification to determine if the JBPDS performs differently for killed agent than it does for live agent.

ASEC (BL1): Classical logistic regression statistical models will be constructed for the probability of detection as a function of concentration and for the probability of identification as a function of concentration for both BG. A statistical test will be performed for both detection and identification to determine if the JBPDS performs differently for killed BG than it does for live BG.

ABT (BL1): Classical logistic regression statistical models will be constructed for the probability of detection as a function of concentration and for the probability of

identification as a function of concentration for 1. wet BG, 2. wet BG with interferent, 3. dry BG, and 4. dry BG with interferent. For each of the 4 cases a statistical test will be performed for both detection and identification to determine if the JBPDS performs differently for killed BG than it does for live BG.

Field (BL1): Classical logistic regression statistical models will be constructed for the probability of detection as a function of concentration and for the probability of identification as a function of concentration for 1. wet BG, 2. wet BG with interferent, 3. dry BG, and 4. dry BG with interferent. For each of the 4 cases a statistical test will be performed for both detection and identification to determine if the JBPDS performs differently for killed BG than it does for live BG.

To test these hypotheses a logistic regression model will be constructed for 19 comparisons of killed versus live agent for both detection and identification. The random component Y_i is binary 0 or 1 for no detection or detection (also no identification or identification). The explanatory variables for this model are agent or simulant concentration, system under test, and live or killed. All models used in hypothesis testing will be evaluated for goodness of fit.

The Detection Model for hypothesis 1 is as follows:

$$\text{logit}(\pi) = \log(\pi / (1- \pi)) = \alpha + \Sigma \alpha_i j_i + \beta_{i1} l_i + \beta_{i2} x_i$$

$$P(\text{detect} \mid x,j,l) = e^{\alpha + \Sigma \alpha j_i + \beta_{i1} l_i + \beta_{i2} x_i} / (1 + e^{\alpha + \Sigma \alpha j_i + \beta_{i1} l_i + \beta_{i2} x_i})$$

Where: π = probability of detect

α = shift parameter

The remaining terms are summed over 1 for CAC, 2 for ASEC or ABT, and 7 for field:

α_i= shape parameter that adjusts a based on the ith system under test

j_i= indicator that identifies the ith system (JPBDS) is under test

l_i = 1 if alive (or not denatured), 0 if dead (or denatured) for the ith system

β_{i1} = live flag shape parameter for the ith system

x_i = concentration for the ith system

β_{i2} = concentration shape parameter for the ith system

The identification model will be the same as above except detection will be replaced with identification.

For this model, hypothesis 1 is now equivalent to:

- Ho: $\beta_{i1} = 0$
- Ha: β_{i1} not equal 0

The test statistic is the Likelihood-ratio test statistic: $-2\log(L_0/L_1) = -2(l_0-l_1)$. Where L_0 is the likelihood function without β_{i1} and L_1 is likelihood function of the full model. This test Statistic is Chi-squared with degrees of freedom equal to the difference in the number of parameters between the two models

Hypothesis 2:

- H_o: JBPDS performance is the same with either live agent or the corresponding live ALO.
- H_a: JBPDS performance is not the same with live agent as it is with the corresponding live ALO.

CAC (BL3): Classical logistic regression statistical models will be constructed for the probability of detection as a function of concentration and for the probability of identification as a function of concentration for each of the following: 1. Spore Bacteria crude, 2. Spore Bacteria washed twice, 3. Vegetative Bacteria, and 4. Virus. For each of the cases a statistical test will be performed for both detection and identification to determine if the JBPDS performs differently for agent than it does for ALO.

To test these hypotheses a logistic regression model will be constructed for 4 comparisons of live ALO versus live agent for both detection and identification. The random component Y_i is binary 0 or 1 for no detection or detection (also no identification or identification). The explanatory variables for this model are agent or simulant concentration, and agent or simulant. All models used in hypothesis testing will be evaluated for goodness of fit.

The Detection Model for hypothesis 2 is as follows:

$$\text{logit}(\pi) = \log(\pi / (1- \pi)) = \alpha + \beta_1 S + \beta_2 x$$

$$P(\text{detect} \mid x,S) = e^{\alpha+\beta_1 S+\beta_1 x} / (1 + e^{\alpha+\beta_1 S+\beta_1 x})$$

Where: π = probability of detect

α = shift parameter

S = 1 if Agent, 0 if ALO

β_1 = agent flag shape parameter

x = concentration

β_2 = concentration shape parameter

The identification model will be the same as above except detection will be replaced with identification.

For this model, hypothesis 2 is now equivalent to:

- Ho: $\beta_1 = 0$
- Ha: β_1 not equal 0

The test statistic is the Likelihood-ratio test statistic: $-2\log(L_0/L_1) = -2(l_0-l_1)$. Where L_0 is the likelihood function without β_1 and L_1 is likelihood function of the full model. This test Statistic is Chi-squared with degrees of freedom equal to the difference in the number of parameters between the two models

Hypothesis 3:

- H_o: JBPDS performance is the same with either killed ALO or live agent.
- H_a: JBPDS performance with killed ALO is different from it's performance with live agent.

CAC (BL3): Classical logistic regression statistical models will be constructed for the probability of detection as a function of concentration and for the probability of identification as a function of concentration for each of the following: spore bacteria, spore bacteria washed twice, vegetative bacteria, and virus. For each of the cases a statistical test will be performed for both detection and identification to determine if the JBPDS performs differently for agent than it does for ALO.

To test these hypotheses a logistic regression model will be constructed for 4 comparisons of live ALO versus live agent for both detection and identification. The random component Yi is binary 0 or 1 for no detection or detection (also no identification

or identification). The explanatory variables for this model are agent or simulant concentration, and agent or simulant. All models used in hypothesis testing will be evaluated for goodness of fit.

The Detection Model for hypothesis 3 is as follows:

$\text{logit}(\pi) = \log(\pi / (1- \pi)) = \alpha + \beta_1 S + \beta_2 x$

$P(\text{ detect} \mid x,S) = e^{\alpha+\beta_1 S+\beta_1 x} / (1 + e^{\alpha+\beta_1 S+\beta_2 x})$

Where: π = probability of detect

α = shift parameter

S = 1 if live agent, 0 if killed ALO

β_1 = agent flag shape parameter

x = concentration

β_2 = concentration shape parameter

The identification model will be the same as above except detection will be replaced with identification.

For this model, hypothesis 3 is now equivalent to:

- Ho: $\beta_1 = 0$
- Ha: β_1 not equal 0

The test statistic is the Likelihood-ratio test statistic: $-2\log(L_0/L_1) = -2(l_0-l_1)$. Where L_0 is the likelihood function without β_1 and L_1 is likelihood function of the full model. This test Statistic is Chi-squared with df equal to the difference in the number of parameters between the two models

If there are statistically significant differences in the statistical tests supporting hypotheses 1 to 3, exploratory data analytical techniques will be used to look for trends.

Conclusions will focus on which agent types can be represented by killed or inactivated ALO.

The Chamber Effect

Hypothesis:

- H_o: JBPDS performance is the same in all chambers (CAC, ASEC, ABT, and the field)
- H_a: JBPDS performance is not the same in all chambers (CAC, ASEC, ABT, and the field)

Classical logistic regression statistical models will be constructed for the probability of detection as a function of concentration and for the probability of identification as a function of concentration for 1. live BG, 2. killed BG , 3. ALO spore bacteria killed crude 4. ALO spore bacteria killed washed twice 5. ALO vegetative bacteria killed ALO, 6. virus killed, and 7. toxoid. For each of the 7 cases a statistical test will be performed for both detection and identification to determine if the JBPDS performs differently in the different chamber in which it is tested.

To test these hypotheses a logistic regression model will be constructed for 7 comparisons of the chamber effect for both detection and identification. The random component Yi is binary 0 or 1 for no detection or detection (also no identification or identification). The explanatory variables for this model are agent or simulant concentration, system under test, chamber type, and agent or simulant. All models used in hypothesis testing will be evaluated for goodness of fit.

The Detection Model for this hypothesis is as follows:

$$\text{logit}(\pi) = \log(\pi / (1- \pi)) = \alpha + \Sigma \alpha_i j_i + \beta_{ic} c_i + \beta_{i2} x_i$$

$$P(\text{ detect} \mid x,j,c) = e^{\alpha + \Sigma \alpha_i j_i + \beta_{ic} c_i + \beta_{i2} x_i} / (1 + e^{\alpha + \Sigma \alpha_i j_i + \beta_{ic} c_i + \beta_{i2} x_i})$$

Where: π = probability of detect

α = shift parameter

The remaining terms are summed over 12 to represent each system under test (1 in the CAC, 2 in both the ASEC and ABT, and 7 in the field)

α_i= shape parameter that adjusts a based on the ith system under test

j_i= indicator that identifies the ith system (JPBDS) is under test

c_i = 1 if CAC, 2 ASEC, 3 if ABT, 4 if field

β_{ic} = live flag shape parameter for the ith system

x_i = concentration for the ith system

β_{i2} = concentration shape parameter for the ith system

The identification model will be the same as above except detection will be replaced with identification.

For this model, this hypothesis is now equivalent to:

- Ho: $\beta_{ic} = 0$
- Ha: β_{ic} not equal 0

The test statistic is the Likelihood-ratio test statistic: $-2\log(L_0/L_1) = -2(l_0-l_1)$. Where L_0 is the likelihood function without β_{i1} and L_1 is likelihood function of the full

model. This test Statistic is Chi-squared with degrees of freedom equal to the difference in the number of parameters between the two models

Heuristic Logistic Regression

Hypothesis:

- H_o: JBPDS performance can be predicted with a model based on logistic regression.
- H_a: JBPDS performance cannot be predicted with a model based on logistic regression.

Heuristic logistic regression models will be constructed for both the detection function and the identification function in JBPDS. The Heuristic logistic regression model is described in chapter 2 in the JBPDS Agent-Simulant Transformations section.

Goodness of fit for each of the heuristic logistic regression models will be judged by:

- Residual plots and other diagnostic plots, and
- Goodness of fit tests.

The heuristic logistic regression models will be rank ordered based on Goodness of fit. Both the quantitative test results and subjective interpretation of plots will influence the rank order. Model attributes such as agent category, ABT vs Field, detection vs identification, how closely the killed ALO represents the living agent, and living vs killed will be related to the rank order based on goodness of fit. Based on the rank ordering and map to model attributes, inferences will be made as to under what

conditions the heuristic logistic regression will be useful in predicting system performance.

Other Models

If the proposed heuristic logistic regression procedure produces models of non-acceptable goodness of fit, alternative methodologies will be proposed. These models may include the addition of a shift parameter and the use of classic logistic regression. Exploratory data analysis across chamber type will facilitate additional model development.

Chapter 4 ALOs as Simulants

This Chapter will focus on how acceptable killed ALOs are as simulants for representing live BWA during biological warfare agent detector field tests. The determination of acceptability is a two-step process. First, if there is no statistical difference between detector performance with killed ALO and detector performance with live BWA then the simulant would be acceptable. Second, if there is statistical difference between detector performances with killed ALO and live BWA, then that difference will be characterized and a determination made as to how it would impact performance in an open air field test. If and only if the impact is minimal, then the simulant will be judged as acceptable. The measures used to judge the impact of the difference in performance in an open air field test were:

- the maximum difference in the expected detector performance between killed ALO and live BWA,
- the concentration range over which a meaningful difference in detector performance occurs, and
- the expected percentage of field trials that would occur in the range over which there is a meaningful difference in detector performance.

The statistical difference in detector performance between most of the killed ALOs and BWA falls on the cusp of statistical significance. Hence, judgment on the impact of the difference in performance in an open air field test is a key portion of this

dissertation. Two concepts aid in putting this judgment in proper context. First although there is a large shift in performance between the CAC and field trials, the relative difference between killed and live challenge material remains quite small. This concept is depicted in figure 5-1 and discussed in chapter 5 in the Comparison of the Intercept and the Live Indicator Estimates section. Second, there is great unexplained variability in open air field trials and concentration alone does not explain a large amount of the variability. This concept is a focus of chapter 6.

This chapter provides results and discussions for each of the following hypotheses for both the detect function and the identification function.

Hypothesis 1:

- H_o: JBPDS performance is the same with killed and live agent (or killed and live simulant).
- H_a: JBPDS performance is different for killed and live agent (or killed and live simulant).

Hypothesis 2:

- H_o: JBPDS performance is the same with live agent or the corresponding live ALO.
- H_a: JBPDS performance is not the same with live agent as it is with the corresponding live ALO.

Hypothesis 3:

- H_o: JBPDS performance is the same with killed ALO and live agent.

- H_a: JBPDS performance with killed ALO is different from its performance with live agent.

Rejecting the null hypothesis in hypothesis 3 provides motivation to develop an agent simulant transformation, which is discussed in chapter five. Hypothesis 1 and 2 provide information on the mechanism of why the null hypothesis in hypothesis 3 is rejected.

The following detection observations are developed in this chapter:

- Killed LE ALO is an acceptable simulant for the detection of LE biological warfare agent. Even though JBPDS detection performance when challenged with live LE biological warfare agent is statistically different from its detection performance when challenged with killed LE ALO simulant (p-value =0.0437), the concentration range of 23 ACPLA over which a meaningful difference occurs is too narrow to have a consequential impact on field test results. During a typical field test the aerosol cloud concentrations range from less than 1 to 16,000 ACPLA. Based on the empirical concentration distribution from the field trials, 11.39% of the challenges fell in this 23 ACPLA range. Based on the logistic regression, the maximum difference in expected detection performance between challenges of LE and killed LE ALO is 0.62.
- Killed NU ALO is an acceptable simulant for the detection of Nu biological warfare agent. Even though JBPDS detection performance when challenged with live Nu biological warfare agent is statistically different from its detection performance when challenged with killed NU ALO simulant (p-

value =0.0234), the concentration range of 55 ACPLA over which a meaningful difference occurs is too narrow to have a consequential impact on field test results. During a typical field test the aerosol cloud concentrations range from less than 1 to 16,000 ACPLA. Based on the empirical concentration distribution from the field trials, 8.9% of the challenges fell in this 55 ACPLA range. Based on the logistic regression, the maximum difference in expected detection performance between challenges of LE and killed LE ALO is 0.95.

- Killed N ALO is an acceptable simulant for the detection of N biological warfare agent. Even though JBPDS detection performance when challenged with live N biological warfare agent is marginally statistically different from its detection performance when challenged with killed N ALO simulant (p-value =0.0846), the concentration range of 7 ACPLA over which a meaningful difference occurs is too narrow to have a consequential impact on field test results. During a typical field test the aerosol cloud concentrations range from less than 1 to 16,000 ACPLA. Few if any of the releases will be at a concentration that produces a difference in detection performance. Based on the empirical concentration distribution from the field trials, 2.18% of the challenges fell in this 7 ACPLA range. Based on the logistic regression, the maximum difference in expected detection performance between challenges of LE and killed LE ALO is 0.48.
- Denatured XR is an acceptable simulant for the detection of XR. JBPDS detection performance when challenged with XR biological warfare agent is

marginally statistically different from its detection performance when challenged with denatured XR simulant (p-value =0.0603). A meaningful difference in detection performance occurs over a range of 172 ACPLA, which seems large. However, based on the empirical concentration distribution from the field trials, only 4.99% of the challenges fell in this 172 ACPLA range. Based on the logistic regression, the maximum difference in expected identification performance between challenges of LE and killed LE ALO is 0.96.

The following identification observations are developed in this chapter:

- Killed LE ALO is an acceptable simulant for LE biological warfare agent identification. There was no difference in JBPDS identification performance when challenged with killed LE ALO from JBPDS identification performance when challenged with LE biological warfare agent (p-value=0.1562).
- Killed NU ALO is not an acceptable simulant for NU biological warfare agent identification. At concentrations in which killed NU ALO was readily identified, JBPDS failed to identify any NU biological warfare agent aerosol challenges.
- Killed N ALO is not an acceptable simulant for N biological warfare agent identification. At concentrations in which N biological warfare agent was readily identified, JBPDS failed to identify any killed N ALO aerosol challenges.
- Denatured XR is an acceptable simulant for the identification of XR biological warfare agent. JBPDS identification performance when challenged

with XR biological warfare agent is statistically different from its identification performance when challenged with denatured XR simulant (p-value =0.0294). A meaningful difference in detection performance occurs over a range of 7,150 ACPLA, which seems large. However, based on the empirical concentration distribution from the field trials, 4.99% of the challenges fell in this 7,150 ACPLA range. Based on the logistic regression, the maximum difference in expected identification performance between challenges of LE and killed LE ALO is 0.96.

Statistical Significance

P-values are provided so that the reader can judge statistical significance based on his or her experience and preference. For this analysis the author generally considered P-values less than 0.05 as evidence of statistical significance. P-values greater than 0.05 and less than or equal 0.1 are considered weak or marginal evidence of statistically significant.

Quasi-complete Separation

The maximum likelihood estimation of the logistic regression model is calculated in an iterative process that normally converges to the maximum likelihood estimate. Usually, there is no problem with the convergence. However, if there is quasi-complete separation of observations, the iterative process may not converge to the maximum likelihood estimate. Quasi-complete separation occurs when the observations of the groups being compared do not overlap or overlap minimally. If quasi-complete separation occurs, then the maximum likelihood estimate and associated statistics are not valid and should not be used. It is often possible to eliminate quasi-complete separation

by eliminating noncritical variables. In this chapter, when quasi-complete separation occurred it was often mitigated by eliminating variables that distinguished between the performances of different systems. In other cases, an evaluation as to the cause of quasi-complete separation became the keystone of the conclusions.

Detection Performance in the CAC with Killed and Live Agent (or Simulant)

Hypotheses 1 for detection will be evaluated in this section. Hypotheses 1 compares the detection performance of JBPDS when challenged with live agent or live simulant with detection performance of JBPDS when challenged with that same agent or simulant after it has been killed. The purpose of this hypothesis is twofold. First, if performance with the killed ALO is different from performance with live agent, it provides a scientific explanation as to if the killing process contributes to the difference in performance. Second, if the killing process resulted in the difference in performance between live agent and its corresponding killed ALO, it provides information that guide changes in the killing process that may improve the ALO simulant.

The following summary of results is developed in this section:

- The null hypothesis in hypothesis 1 is rejected for the follow pairs of agent or simulant and their corresponding killed agent or simulant: live washed N compared to killed washed N (p-value=0.0367), and live NU and killed NU (p-value=0.0718). That is to say, for washed N and NU JBPDS detection performance when challenged with live agent is statistically different from its performance when challenged with the corresponding killed agent.

- The null hypothesis in hypothesis 1 is not rejected for the follow pairs of agent or simulant and their corresponding killed agent or simulant: live BG and killed BG (p-value=0.1727), live LE and killed LE (p-value=0.4564), live LE ALO and killed LE ALO (p-value=0.6447), live N ALO and killed N ALO (p-value=0.4806). That is to say for these agents and simulants there is no evidence to suggest that JBPDS detection performance when challenged with live agent or simulant is different from its performance when challenged with the corresponding killed agent.

BG CAC detection results are based on 43 BG challenges at various concentrations of which 25 were detected and 18 were not detected.

The JBPDS BG detection model fit statistics for the CAC are summarized in table 4-1. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-Square of 0.79 indicates that there is predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 11.11 with 8 degrees of freedom which produces a p-value of 0.19. The deviance goodness of fit statistic is 19.74 with 37 degrees of freedom and a p-value of 0.99. Neither goodness of fit tests are statistically significant which suggests that the model is not a bad fit.

Table 4-1 BG CAC Detection Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	60.466	25.741
SC	62.227	31.025
-2 Log L	58.466	19.741
R-Square 0.5937	Max-rescaled R-Square 0.7987	

Testing Global Null Hypothesis: All coefficients=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	38.7248	2	<.0001
Score	21.3299	2	<.0001
Wald	8.0011	2	0.0183

The intercept and the natural log of concentration both contribute to this model (p-values of 0.0057 and 0.0051 respectively). As can be seen in table 4-2, there is little if any evidence that the detection performance of JBPDS when challenged with live BG is different from when it is challenged with dead BG. We conclude that in the CAC, JBPDS detection performance is the same with killed and live BG (P-value=0.1727).

Table 4-2 BG CAC Detection Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	-22.6325	8.1899	7.6368	0.0057
Natural Log of Concentration	1	6.2663	2.2385	7.8366	0.0051
LIVE Indicator	1	-0.9719	0.7128	1.8591	0.1727

LE CAC detection results are based on 48 LE challenges at various concentrations.

The JBPDS LE detection model fit statistics for the CAC are summarized in table 4-3. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-Square of 0.89 indicates that there is predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 0.58 with 5 degrees of freedom which produces a p-value of 0.99. The deviance goodness of fit statistic is 9.23 with 43 degrees of freedom and a p-value of 0.99. Neither goodness of fit tests are statistically significant which suggests that the model is not a bad fit.

Table 4-3 LE CAC Detection Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	53.674	15.226
SC	55.545	20.840
-2 Log L	51.674	9.226
R-Square 0.5870	Max-rescaled R-Square 0.8904	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	42.4475	2	<.0001
Score	17.6651	2	0.0001
Wald	3.5953	2	0.1657

The intercept and the natural log of concentration both contribute to this model (p-values of 0.0757 and 0.0763 respectively). As can be seen in table 4-4, there is little if any evidence that the detection performance of JBPDS when challenged with live LE is different from when it is challenged with dead LE. We conclude that in the CAC, JBPDS detection performance is the same with killed and live LE (P-value=0.4564).

Table 4-4 LE CAC Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	22.3768	3.1549	0.0757
Natural Log of Concentration	1	7.9333	3.1422	0.0763
LIVE Indicator	1	1.1649	0.5547	0.4564

LE ALO CAC detection results are based on 61 LE ALO challenges at various concentrations.

The JBPDS LE detection model fit statistics for the CAC are summarized in table 4-5. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-square of 0.74 indicates that there is predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 3.5 with 5 degrees of freedom which produces a p-value of 0.64. The deviance goodness of fit statistic is 20.93 with 55 degrees of freedom and a p-value of 0.99. Neither goodness of fit tests are statistically significant which suggests that the model is not a bad fit.

Table 4-5 LE ALO CAC Detection Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	65.203	29.704
SC	67.314	36.037
-2 Log L	63.203	23.704
R-Square 0.4767	Max-rescaled R-Square 0.7388	
Testing Global Null Hypothesis: All Coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	39.4987	2	<.0001
Score	18.1410	2	0.0001
Wald	7.2399	2	0.0268

The intercept and the natural log of concentration both contribute to this model (p-values of 0.0076 and 0.0075 respectively). As can be seen in table 4-6, there is little if any evidence that the detection performance of JBPDS when challenged with live LE ALO is different from when it is challenged with dead LE ALO. We conclude that in the CAC, JBPDS detection performance is the same with killed and live LE ALO (P-value=0.6447).

Table 4-6 LE ALO CAC Detection Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	8.3819	7.1208	0.0076
Natural Log of53 Concentration	1	2.3385	7.1499	0.0075
LIVE Indicator	1	0.5328	0.2127	0.6447

N CAC detection results are based on 44 N challenges at various concentrations.

The N model which is depicted in tables 4-7 and 4-8 includes an intercept, the natural log of the concentration, and an indicator parameter for living or killed N. Regrettably, there was quasi-complete separation of data points in this model and hence, the maximum likelihood estimate may not exist. This model is not suited for analysis.

Table 4-7 N CAC Detection Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	53.564	6.178
SC	55.348	11.531
-2 Log L	51.564	0.178
R-Square 0.6890	Max-rescaled R-Square 0.9982	
Testing Global Null Hypothesis: All Coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	51.3857	2	<.0001
Score	25.9496	2	<.0001
Wald	1.4918	2	0.4743

Table 4-8 N CAC Detection Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	124.8	1.4868	0.2227
Natural Log of Concentration	1	31.2797	1.4870	0.2227
LIVE N Indicator	1	6.4823	0.9554	0.3283

The data set on N detection in the CAC is not suitable to support a comparison of JBPDS detection performance differences between live and killed N.

N ALO CAC detection results are based on 42 N ALO challenges at various concentrations.

The JBPDS N ALO detection model fit statistics for the CAC are summarized in table 4-9. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-square of 0.93 indicates that there is predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 1.0 with 8 degrees of freedom which produces a p-value of 0.99. The deviance goodness of fit

statistic is 8.21 with 36 degrees of freedom and a p-value of 0.99. Neither goodness of fit tests are statistically significant which suggests that the model is not a bad fit.

Table 4-9 N ALO CAC Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	59.364	14.212
SC	61.102	19.425
-2 Log L	57.364	8.212
R-Square 0.6897	Max-rescaled R-Square 0.9260	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	49.1521	2	<.0001
Score	29.9096	2	<.0001
Wald	3.1670	2	0.2053

The intercept and the natural log of concentration both contribute to this model (p-values of 0.0777 and 0.0752 respectively). As can be seen in table 4-10, there is little if any evidence that the detection performance of JBPDS when challenged with live N ALO is different from when it is challenged with dead N ALO. We conclude that in the CAC, JBPDS detection performance is the same with killed and live N ALO (P-value=0.4806).

Table 4-10 N ALO CAC Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	29.5266	3.1124	0.0777
Natural Log of Concentration	1	7.7348	3.1662	0.0752
LIVE Indicator	1	3.4918	0.4975	0.4806

Washed N ALO CAC detection results are based on 36 N ALO challenges at various concentrations.

The JBPDS washed N ALO detection model fit statistics for the CAC are summarized in table 4-11. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-square of 0.85 indicates that there is predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 0.79 with 5 degrees of freedom which produces a p-value of 0.98. The deviance goodness of fit statistic is 8.79 with 27 degrees of freedom and a p-value of 0.99. Neither goodness of fit tests are statistically significant which suggests that the model is not a bad fit.

Table 4-11 Washed N ALO CAC Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	47.829	17.559
SC	49.413	22.309
-2 Log L	45.829	11.559
R-Square 0.6140	Max-rescaled R-Square 0.8528	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	34.2702	2	<.0001
Score	17.5086	2	0.0002
Wald	5.1180	2	0.0774

The intercept and the natural log of concentration both contribute to this model (p-values of 0.0301and 0.0290 respectively). As can be seen in table 4-12, there is evidence that the detection performance of JBPDS when challenged with live washed N ALO is different from when it is challenged with dead washed N ALO. We conclude that in the CAC, JBPDS detection performance is different for killed and live washed N ALO (P-value=0.0367).

Table 4-12 Washed N ALO CAC Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	36.7117	4.7063	0.0301
Natural Log of Concentration	1	9.7516	4.7670	0.0290
LIVE Indicator	1	1.3218	4.3644	0.0367

Washed NU ALO CAC detection results are based on 48 NU ALO challenges at various concentrations.

The JBPDS NU ALO detection model fit statistics for the CAC are summarized in table 5-13. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-square of 0.98 indicates that there is predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 1.02 with 5 degrees of freedom which produces a p-value of 0.96. The deviance goodness of fit statistic is 2.13 with 38 degrees of freedom and a p-value of 0.99. Neither goodness of fit tests are statistically significant which suggests that the model is not a bad fit.

Table 4-13 NU ALO CAC Detection Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	63.105	8.131
SC	64.977	13.744
-2 Log L	61.105	2.131
R-Square 0.7073		Max-rescaled R-Square 0.9824
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF Pr > ChiSq
Likelihood Ratio	58.9748	2 <.0001
Score	15.2428	2 0.0005
Wald	3.5626	2 0.1684

The intercept and the natural log of concentration both contribute to this model (p-values of 0.0614 and 0.0610 respectively). As can be seen in table 4-14, there is evidence that the detection performance of JBPDS when challenged with live NU ALO is different from when it is challenged with dead NU ALO. We conclude that in the CAC, JBPDS detection performance is different for killed and live NU ALO (P-value=0.0718).

Table 4-14 NU ALO CAC Detection Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	50.7577	3.5002	0.0614
Natural Log of Concentration	1	14.9420	3.5102	0.0610
LIVE Indicator	1	7.3614	3.2415	0.0718

Detection Performance in the CAC with Live Agent and Live ALO

Hypotheses 2 for detection will be evaluated in this section. Hypotheses 2 compares the detection performance of JBPDS when challenged with live agent with detection performance of JBPDS when challenged with the corresponding live ALO. The purpose of this hypothesis is twofold. First, if performance with a killed ALO is different from performance with live agent, it provides a scientific explanation as to if the basic difference between agent and its corresponding ALO contributed to the difference in performance. Second, if there are fundamental differences in detection performance of JBPDS when challenged with live agent and its corresponding live ALO, then selecting a different ALO could improve the quality of the ALO simulant.

The following summary of results is developed in this section:

- The null hypothesis in hypothesis 2 is rejected for the follow pairs of live agent and their corresponding live ALO: live LE and live LE ALO (p-value=0.0335), live NU and live NU ALO (p-value=0.0287), and washed live N and washed live N ALO (p-value=0.0552). That is to say, for LE, NU, and washed N JBPDS detection performance when challenged with live agent is statistically different from its performance when challenged with the corresponding live ALO.
- The null hypothesis in hypothesis 2 is not rejected for live unwashed N and live unwashed N ALO (p-value=0.9726). That is to say for live unwashed N and live unwashed N ALO there is no evidence to suggest that JBPDS detection performance when challenged with live agent is different from its performance when challenged with the corresponding live ALO.

Live LE and live LE ALO CAC detection results are based on 59 challenges at various concentrations.

The JBPDS Live LE and live LE ALO detection model fit statistics for the CAC are summarized in table 4-15. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-square of 0.79 indicates that there is predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 1.4740 with 6 degrees of freedom which produces a p-value of 0.96. The deviance goodness of fit statistic is 17.29 with 54 degrees of freedom and a p-value of 0.99. Neither goodness of fit tests are statistically significant which suggests that the model is not a bad fit.

Table 4-15 Live LE versus Live LE ALO Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	58.760	23.287
SC	60.838	29.520
-2 Log L	56.760	17.287
R-Square 0.4878	Max-rescaled R-Square 0.7895	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	39.4733	2	<.0001
Score	19.0112	2	<.0001
Wald	6.7062	2	0.0350

The intercept and the natural log of concentration both contribute to this model (p-values of 0.0093 and 0.0096 respectively). As can be seen in table 4-16, there is evidence that the detection performance of JBPDS when challenged with Live LE is different from when it is challenged with Live LE ALO. We conclude that in the CAC, JBPDS detection performance is not the same with live LE as it is with the corresponding live LE ALO (P-value=0.0335).

Table 4-16 Live LE versus Live LE ALO Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	7.8058	6.7681	0.0093
Natural Log of Concentration	1	2.4730	6.7062	0.0096
LE or LE ALO Indicator	1	1.1028	4.5208	0.0335

Live NU and live NU ALO CAC detection results are based on 60 challenges at various concentrations.

The JBPDS Live NU and live NU ALO detection model fit statistics for the CAC are summarized in table 4-17. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-square of 0.93 indicates that there is predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 0.5996 with 7 degrees of freedom which produces a p-value of 0.99. The deviance goodness of fit statistic is 9.55 with 52 degrees of freedom and a p-value of 0.99. Neither goodness of fit tests are statistically significant which suggests that the model is not a bad fit.

Table 4-17 Live NU versus Live NU ALO Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	76.920	15.548
SC	79.014	21.831
-2 Log L	74.920	9.548
R-Square 0.6636	Max-rescaled R-Square 0.9306	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	65.3719	2	<.0001
Score	25.2071	2	<.0001
Wald	6.0016	2	0.0497

The intercept and the natural log of concentration both contribute to this model (p-values of 0.0169 and 0.0161 respectively). As can be seen in table 4-18, there is evidence that the detection performance of JBPDS when challenged with live NU is different from when it is challenged with live NU ALO. We conclude that in the CAC, JBPDS detection performance is not the same with live LE as it is with the corresponding live LE ALO (P-value=0.0287).

Table 4-18 Live NU versus Live NU ALO Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	22.7871	5.7016	0.0169
Natural Log of Concentration	1	5.0654	5.7870	0.0161
NU or NU ALO Indicator	1	3.3751	4.7863	0.0287

Live N and live N ALO CAC detection results are based on 50 challenges at various concentrations.

The JBPDS Live N and live N ALO detection model fit statistics for the CAC are summarized in table 4-19. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-square of 1.00 indicates that there is predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 0.0142 with 3 degrees of freedom which produces a p-value of 0.99. The deviance goodness of fit statistic is 0.26 with 43 degrees of freedom and a p-value of 0.99. Neither goodness of fit tests are statistically significant which suggests that the model is not a bad fit.

Table 4-19 Live N versus Live N ALO Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	67.342	6.257
SC	69.254	11.993
-2 Log L	65.342	0.257
R-Square 0.7279	Max-rescaled R-Square 0.9981	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	65.0848	2	<.0001
Score	36.7310	2	<.0001
Wald	1.4296	2	0.4893

Neither the intercept nor the natural log of concentration obviously contribute to this model (p-values of 0.3004 and 0.2319 respectively). As can be seen in table 4-20, there is no evidence that the detection performance of JBPDS when challenged with live N is different from when it is challenged with live N ALO. It is some what inconsistent that table 4-19 indicates that at least one of the coefficients is non zero, but table 4-20 fails to identify any coefficient as being statistically different from zero. We conclude that in the CAC, JBPDS detection performance is the same with live N as it is with the corresponding live N ALO (P-value=0.9726).

Table 4-20 Live N versus Live N ALO Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	115.8	1.0724	0.3004
Natural Log of Concentration	1	25.7731	1.4289	0.2319
N or N ALO Indicator	1	60.6789	0.0012	0.9726

Washed live N and washed live N ALO CAC detection results are based on 36 challenges at various concentrations.

The JBPDS washed live N and washed live N ALO detection model fit statistics for the CAC are summarized in table 4-21. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-square of 0.88 indicates that there is predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 1.2640 with 5 degrees of freedom which produces a p-value of 0.94. The deviance goodness of fit

statistic is 7.10 with 30 degrees of freedom and a p-value of 0.99. Neither goodness of fit tests are statistically significant which suggests that the model is not a bad fit.

Table 4-21 Washed Live N versus Washed Live N ALO Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	47.829	15.869
SC	49.413	20.620
-2 Log L	45.829	9.869
R-Square 0.6317	Max-rescaled R-Square 0.8774	
Testing Global Null Hypothesis: All Coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	35.9600	2	<.0001
Score	17.4587	2	0.0002
Wald	5.0471	2	0.0802

The intercept and the natural log of concentration both contribute to this model (p-values of 0.0247 and 0.0247 respectively). As can be seen in table 4-22, there is evidence that the detection performance of JBPDS when challenged with washed live N is different from when it is challenged with washed live N ALO. We conclude that in the CAC, JBPDS detection performance is not the same with washed live N as it is with the corresponding washed live N ALO (P-value=0.0552).

Table 4-22 Washed Live N versus Washed Live N ALO Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	34.0616	5.0419	0.0247
Natural Log of Concentration	1	8.1200	5.0432	0.0247
Washed N or washed N ALO Indicator	1	3.0822	3.6754	0.0552

Detection Performance in the CAC with Live LE and Killed LE ALO

This section will provide an evaluation of hypothesis 3 for live LE and killed LE ALO detection in the CAC. This section will provide insight to determine if JBPDS detection performance when challenged with live LE is the same as when it is challenged with killed LE ALO. If not the same then determine the magnitude of that difference and how would it impact a field test. Since hypothesis 3 requires the use of actual biological warfare agent, the CAC is the only chamber where this hypothesis can be tested. The ultimate goal of this section is to determine if killed LE ALO is an acceptable simulant for live LE.

The section conclusion is that killed LE ALO is an acceptable simulant for the detection of LE biological warfare agent. Even though JBPDS detection performance when challenged with live LE biological warfare agent is statistically different from its detection performance when challenged with killed LE ALO simulant (p-value =0.0437), the consideration range of 23 ACPLA over which a meaningful difference occurs is to narrow to have a consequential impact on field test results. During a typical field test the aerosol cloud concentrations range from less than 1 to 16,000 ACPLA. Few if any of the releases will be at a concentration that produces a difference in detection performance.

Live LE and Killed LE ALO CAC detection results are based on 62 challenges at various concentrations.

The JBPDS live LE and Killed LE ALO detection model fit statistics for the CAC are summarized in table 4-23. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-square of 0.81 indicates that there is predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 0.3962 with 6 degrees of freedom which produces a p-value of 0.99. The deviance goodness of fit statistic is 14.50 with 56 degrees of freedom and a p-value of 0.99. Neither goodness of fit tests is statistically significant which suggests that the model is not a bad fit.

Table 4-23 Live LE versus Killed LE ALO Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	62.925	23.278
SC	65.052	29.660
-2 Log L	60.925	17.278
R-Square 0.5054	Max-rescaled R-Square 0.8077	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF Pr > ChiSq
Likelihood Ratio	43.6461	2 <.0001
Score	20.5232	2 <.0001
Wald	5.9162	2 0.0519

The intercept and the natural log of concentration both contribute to this model (p-values of 0.0159 and 0.0161 respectively). As can be seen in table 4-24, there is evidence that the detection performance of JBPDS when challenged with live LE is different from when it is challenged with killed LE ALO. We conclude that in the CAC, JBPDS detection performance with killed LE ALO is different from its performance with live LE agent (P-value=0.0437).

Table 4-24 Live LE versus Killed LE ALO Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	9.7575	5.8085	0.0159
Natural Log of Concentration	1	3.0434	5.7941	0.0161
Live LE or Killed LE ALO Indicator	1	1.4886	4.0665	0.0437

As seen in table 4-25, the difference in JBPDS detection performance is caused by an inherent difference in the detection performance of live LE and Live LE ALO (P-value=0.0335). The act of killing LE or LE ALO has no statistical significance on JBPDS detection performance (P-value=0.4565 and P-value=0.6447 respectively).

Table 4-25 LE Agent and Simulant Summery of P-values

Comparison	Source	P-value
Live LE versus Killed LE	Table 4-4	0.4564
Live LE ALO versus Killed LE ALO	Table 4-6	0.6447
Live LE versus Live LE ALO	Table 4-16	0.0335
Live LE versus Killed LE ALO	Table 4-24	0.0437

The initial thought is that since the detection performance of JBPDS with live LE is statistically different from its performance with killed LE ALO (P-value=0.0437), that killed ALO should not be used as a simulant for live LE. However, before that conclusion is reached and codified it is of interest to calculate the magnitude of the difference in JBPDS detection performance caused by these different challenges. If the difference is small enough, then even though it is statistically significant, LE ALO could still be an adequate simulant. The magnitude of that difference will be examined in the remainder of this section.

The magnitude of the difference in JBPDS detection performance in the CAC between live LE and killed LE ALO will be estimated from the logistic regression model based on the different concentrations of field challenge material.

The following observations are based on a comparison of predicted JBPDS detection performance against live LE with the predicted JBPDS detection performance against killed LE ALO (see Figure 4-1):

- The use of Killed LE ALO as a stimulant for LE will result in either accurate estimations or underestimating JBPDS detection performance.
- At higher or lower concentrations the detection performance of JBPDS when challenged with killed LE ALO and live LE are equivalent.
- The difference in the probability of detection between live LE and killed LE ALO never exceeds 0.63.
- The difference in the probability of detection between live LE and killed LE ALO exceeds 0.60 over a range of 5 ACPLA
- The difference in the probability of detection between live LE and killed LE ALO exceeds 0.50 over a range of 11 ACPLA
- The difference in the probability of detection between live LE and killed LE ALO exceeds 0.40 over a range of 14.5 ACPLA
- The difference in the probability of detection between live LE and killed LE ALO exceeds 0.3 over a range of 20 ACPLA
- The difference in the probability of detection between live LE and killed LE ALO exceeds 0.2 over a range of 23 ACPLA

- The difference in the probability of detection between live LE and killed LE ALO exceeds 0.1 over a range of 33 ACPLA
- The difference in the probability of detection between live LE and killed LE ALO exceeds .05 over a range of 47 ACPLA

Clearly there is a relatively narrow range of concentration over which the simulant, killed LE ALO produces JBPDS detection performance which is different from that produced by the agent, LE.

Data from 504 field simulant challenges was used to construct an empirical distribution of challenge concentration. From this the probability of a field concentration was multiplied by the expected difference in performance between LE and killed LE ALO. These products were summed to produce the expected difference and are depicted in table 4-26. Clearly, over most of the concentration range of the field trials there is little or no difference in performance between live LE and killed LE ALO.

Table 4-26 Live LE and Killed LE ALO Expected Detection Difference

Difference in predicted detection performance between Agent and Simulant	**Fraction of Trials with Simulant Concentration that would produce the Difference**	**Expected Difference**
0.60-0.63	0.0160	0.06737
0.50-0.5999	0.0140	
0.40-0.4999	0.0179	
0.30-0.3999	0.0400	
0.20-0.2999	0.0260	
0.10-0.1999	0.0675	
0.05-0.9999	0.0477	
0.00-0.0499	0.8069	

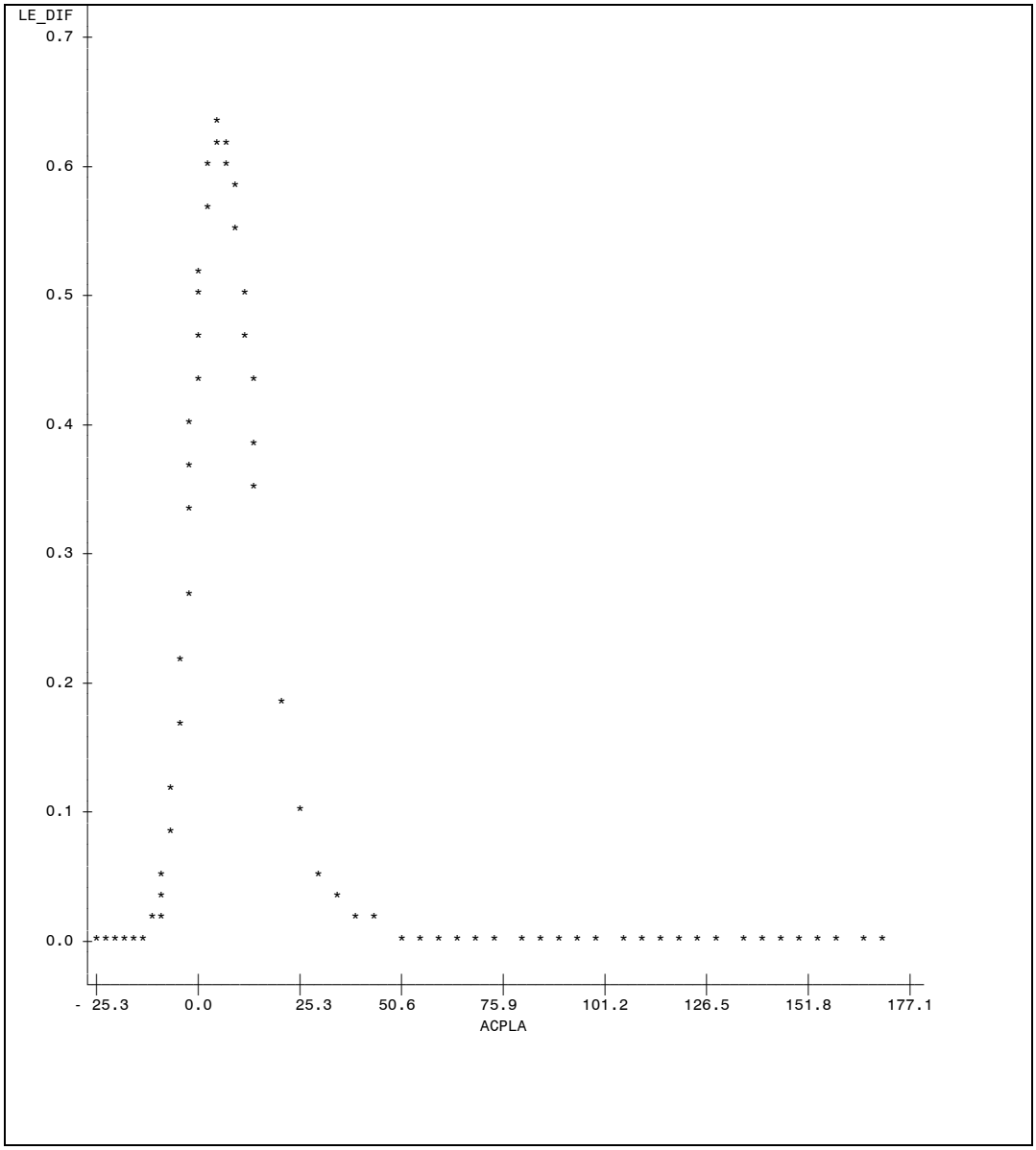


Figure 4-1 JBPDS Detection Performance. In this plot, LE_DIF = P(Detect LE| Concentration)- P(Detect killed LE ALO| Concentration). Concentration or ACPLA has been shifted to create an unclassified figure.

Detection Performance in the CAC with Live NU and Killed NU ALO

This section will provide an evaluation of hypothesis 3 for live NU and killed NU ALO detection in the CAC. This section will provide insight to determine if JBPDS detection performance when challenged with live NU is the same as when it is challenged with killed NU ALO. If not the same then determine the magnitude of that difference and how would it impact a field test. Since hypothesis 3 requires the use of actual biological warfare agent, the CAC is the only chamber where this hypothesis can be tested. The ultimate goal of this section is to determine if killed NU ALO is an acceptable simulant for live NU.

The section conclusion is that Killed Nu ALO is an acceptable simulant for the detection of Nu biological warfare agent. Even though JBPDS detection performance when challenged with live Nu biological warfare agent is statistically different from its detection performance when challenged with killed Nu ALO simulant (p-value =0.0234), the consideration range of 55 ACPLA over which a meaningful difference occurs is to narrow to have a consequential impact on field test results. During a typical field test the aerosol cloud concentrations range from less than 1 to 16,000 ACPLA. Few if any of the releases will be at a concentration that produces a difference in detection performance.

Live NU and Killed NU ALO CAC detection results are based on 72 challenges at various concentrations.

The JBPDS live NU and Killed NU ALO detection model fit statistics for the CAC are summarized in table 4-27. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-square of 0.93 indicates that there is predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 0.2566 with 6 degrees of freedom which produces a p-value of 0.99. The deviance goodness of fit statistic is 11.08 with 61 degrees of freedom and a p-value of 0.99. Neither goodness of fit tests are statistically significant which suggests that the model is not a bad fit.

Table 4-27 Live NU versus Killed NU ALO Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	92.209	17.083
SC	94.485	23.913
-2 Log L	90.209	11.083
R-Square 0.6668	Max-rescaled R-Square 0.9334	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	79.1257	2	<.0001
Score	28.9080	2	<.0001
Wald	6.0975	2	0.0474

The intercept and the natural log of concentration both contribute statistically to this model (p-values of 0.0175 and 0.0169 respectively). As can be seen in table 4-28, there is evidence that the detection performance of JBPDS when challenged with live NU is different from when it is challenged with killed NU ALO. We conclude that in the CAC, JBPDS detection performance with killed NU ALO is different from its performance with live LE agent (P-value=0.0234).

Table 4-28 Live NU versus Killed NU ALO Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	20.7968	5.6487	0.0175
Natural Log of Concentration	1	5.1670	5.7109	0.0169
Live NU or Killed NU ALO Indicator	1	5.8258	5.1356	0.0234

As seen in table 4-29, the difference in JBPDS detection performance is caused by an inherent difference in the detection performance of live NU and Live NU ALO (P-value=0.0287). In addition, the act of killing NU ALO also has a statistical significance on JBPDS detection performance (P-value=0.0718).

Table 4-29 NU Agent and Simulant Summery of P-values

Comparison	Source	P-value
Live NU ALO versus Killed NU ALO	Table 4-14	0.0718
Live NU versus Live NU ALO	Table 4-18	0.0287
Live NU versus Killed NU ALO	Table 4-31	0.0234

The initial thought is that since the detection performance of JBPDS with live NU is statistically different from its performance with killed NU ALO (P-value=0.0234), that killed ALO should not be used as a simulant for live LE. However, before that conclusion is reached and codified it is of interest to calculate the magnitude of the difference in JBPDS detection performance caused by these different challenges. If the difference is small enough, then even though it is statistically significant, NU ALO could still be an adequate simulant. The magnitude of that difference will be examined in the remainder of this section.

The magnitude of the difference in JBPDS detection performance between live NU and killed NU ALO will be estimated from the logistic regression model over different concentrations of challenge material.

The following observations are based on a comparison of predicted JBPDS detection performance against live NU with the predicted JBPDS detection performance against killed NU ALO (see Figure 4-2):

- The use of Killed NU ALO as a stimulant for NU will result in either accurate estimations or overestimating JBPDS detection performance.
- At higher or lower concentrations the detection performance of JBPDS when challenged with killed NU ALO and live NU are equivalent.
- The difference in the probability of detection between live NU and killed NU ALO never exceeds 0.95.
- The difference in the probability of detection between live NU and killed NU ALO exceeds 0.90 over a range of 14.5 ACPLA
- The difference in the probability of detection between live NU and killed NU ALO exceeds 0.80 over a range of 24.5 ACPLA
- The difference in the probability of detection between live NU and killed NU ALO exceeds 0.70 over a range of 30.0 ACPLA
- The difference in the probability of detection between live NU and killed NU ALO exceeds 0.60 over a range of 35 ACPLA
- The difference in the probability of detection between live NU and killed NU ALO exceeds 0.50 over a range of 39.5 ACPLA

- The difference in the probability of detection between live NU and killed NU ALO exceeds 0.40 over a range of 44.0 ACPLA
- The difference in the probability of detection between live NU and killed NU ALO exceeds 0.30 over a range of 48.5 ACPLA
- The difference in the probability of detection between live NU and killed NU ALO exceeds 0.20 over a range of 55.0 ACPLA
- The difference in the probability of detection between live NU and killed NU ALO exceeds 0.10 over a range of 64.5 ACPLA
- The difference in the probability of detection between live NU and killed NU ALO exceeds 0.050 over a range of 73.5 ACPLA

Clearly there is a relatively narrow range of concentration over which the simulant, killed NU ALO produces JBPDS detection performance which is different from that produced by the agent, NU.

Data from 504 field simulant challenges was used to construct an empirical distribution of challenge concentration. From this the probability of a field concentration was multiplied by the expected difference in performance between NU and killed NU ALO. These products were summed to produce the expected difference and are depicted in table 4-30. Clearly, over most of the concentration range of the field trials there is little or no difference in performance between live NU and killed NU ALO.

Table 4-30 Live NU and Killed NU ALO Expected Detection Difference

Difference in predicted detection performance between Agent and Simulant	**Fraction of Trials with Simulant Concentration that would produce the Difference**	**Expected Difference**
0.90-0.95	0.0233	0.08974
0.80-0.8999	0.0258	
0.70-0.7999	0.0100	
0.60-0.6999	0.0079	
0.50-0.5999	0.0060	
0.40-0.4999	0.0040	
0.30-0.3999	0.0060	
0.20-0.2999	0.0060	
0.10-0.1999	0.0140	
0.05-0.0599	0.0079	
0.00-0.0499	0.8891	

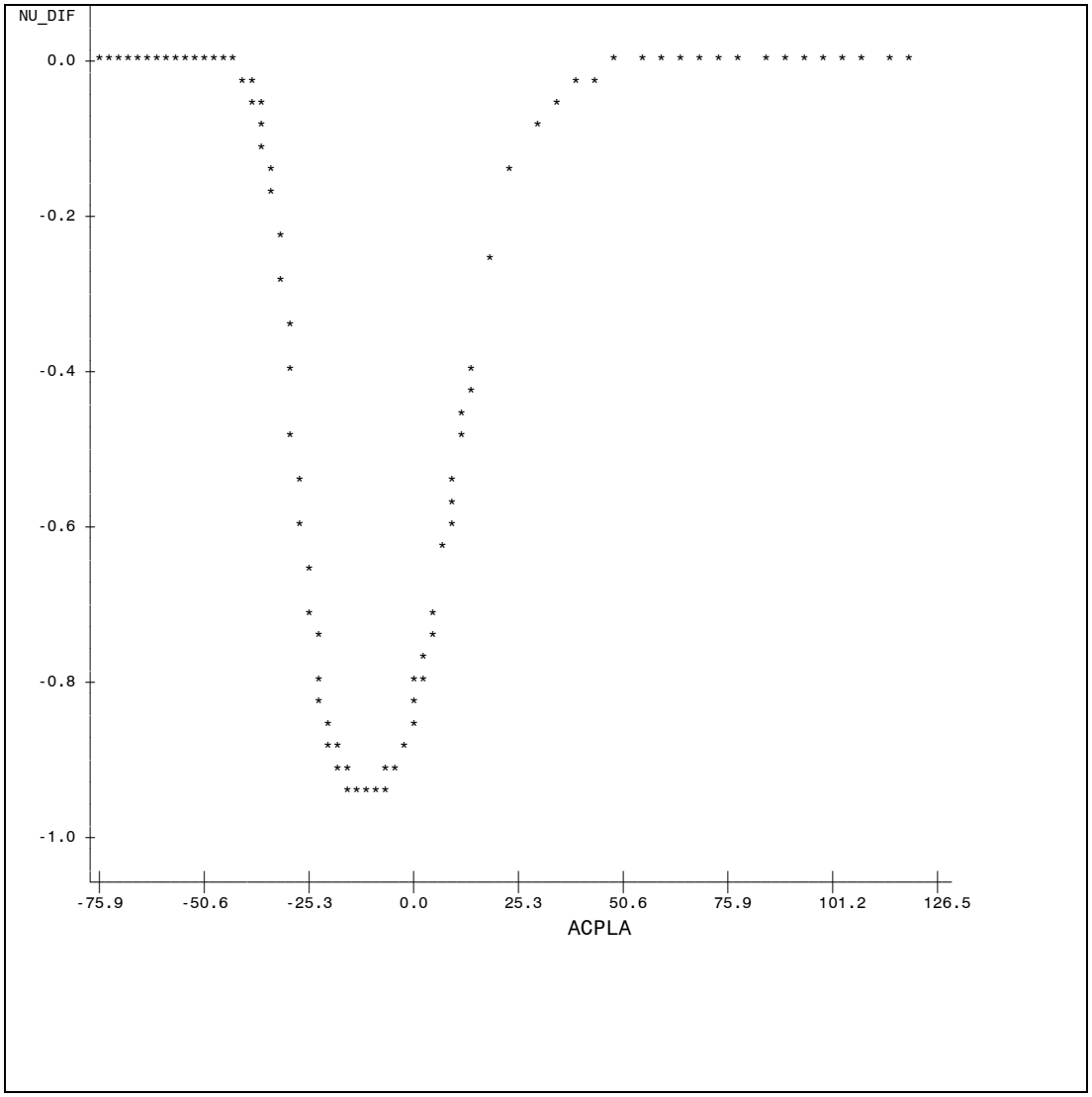


Figure 4-2 JBPDS Detection Performance. In this plot, NU_DIF = P(Detect live NU| Concentration)- P(Detect killed NU ALO| Concentration). Concentration or ACPLA has been shifted to create an unclassified figure.

Detection Performance in the CAC with Live N and Killed N ALO

This section will provide an evaluation of hypothesis 3 for live N and killed N ALO detection in the CAC. This section will provide insight to determine if JBPDS detection performance when challenged with live N is the same as when it is challenged with killed N ALO. If not the same then determine the magnitude of that difference and how would it impact a field test. Since hypothesis 3 requires the use of actual biological warfare agent, the CAC is the only chamber where this hypothesis can be tested. The ultimate goal of this section is to determine if killed N ALO is an acceptable simulant for live N.

The section conclusion is that Killed N ALO is an acceptable simulant for the detection of N biological warfare agent. Even though JBPDS detection performance when challenged with live N biological warfare agent is marginally statistically different from its detection performance when challenged with killed N ALO simulant (p-value =0.0846), the consideration range of 7 ACPLA over which a meaningful difference occurs is to narrow to have a consequential impact on field test results. During a typical field test the aerosol cloud concentrations range from less than 1 to 16,000 ACPLA. Few if any of the releases will be at a concentration that produces a difference in detection performance.

Live N and Killed N ALO CAC detection results are based on 44 challenges at various concentrations.

The JBPDS live N and Killed N ALO detection model fit statistics for the CAC are summarized in table 4-31. Clearly, at least one coefficient in the model is not equal

to zero. A rescaled R-square of 0.89 indicates that there is predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 0.3033 with 6 degrees of freedom which produces a p-value of 0.99. The deviance goodness of fit statistic is 9.47 with 40 degrees of freedom and a p-value of 0.99. Neither goodness of fit tests are statistically significant which suggests that the model is not a bad fit.

Table 4-31 Live N versus Killed N ALO Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	53.564	15.472
SC	55.348	20.825
-2 Log L	51.564	9.472
R-Square 0.6158	Max-rescaled R-Square 0.8922	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	42.0917	2	<.0001
Score	21.9636	2	<.0001
Wald	4.8048	2	0.0905

The intercept and the natural log of concentration both contribute to this model (p-values of 0.0301 and 0.0308 respectively). As can be seen in table 4-32, there is evidence that the detection performance of JBPDS when challenged with live N is different from when it is challenged with killed N ALO. We conclude that in the CAC, JBPDS detection performance with killed N ALO is different from its performance with live N agent (P-value=0.0846).

Table 4-32 Live N versus Killed N ALO Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	31.9886	4.7057	0.0301
Natural Log of Concentration	1	8.6341	4.6617	0.0308
Live N or Killed N ALO Indicator	1	1.2543	2.9737	0.0846

As seen in table 4-33, the difference in JBPDS detection performance is more pronounced and statistically significant for pairs that have been washed than for unwashed pairs. This suggests that the differences in detection performance are reduced because of the media that clings to N. It is clear that the detection performance JBPDS for live washed agent is statistically different from the live washed ALO (P-value=0.0552).

Table 4-33 N Agent and Simulant Summery of P-values

Comparison	Source	P-value
Live N versus Killed N	Table 4-8	0.3283
Live N ALO versus Killed N ALO	Table 4-10	0.4806
Live washed N ALO versus Killed Washed N ALO	Table 4-12	0.0367
Live N versus Live N ALO	Table 4-20	0.9726
Live washed N versus Live washed N ALO	Table 4-22	0.0552
Live N versus Killed N ALO	Table 4-38	0.0846

The initial thought is that since the detection performance of JBPDS with live N is statistically different from its performance with killed N ALO (P-value=0.0846), that killed ALO should not be used as a simulant for live N. However, before that conclusion is reached and codified it is of interest to calculate the magnitude of the difference in JBPDS detection performance caused by these different challenges. If the difference is small enough, then even though it is statistically significant, N ALO could still be an

adequate simulant. The magnitude of that difference within the CAC will be examined in the remainder of this section. The magnitude of that difference will then be extrapolated to the field in the next chapter.

The magnitude of the difference in JBPDS detection performance in the CAC between live N and killed N ALO will be estimated from the logistic regression model over different concentrations of challenge material.

The following observations are based on a comparison of predicted JBPDS detection performance against live N in the CAC with the predicted JBPDS detection performance against killed N ALO in the CAC (see Figure 4-3):

- The use of Killed N ALO as a stimulant for N will result in either accurate estimations or underestimating JBPDS detection performance.
- At higher or lower concentrations the detection performance of JBPDS when challenged with killed N ALO and live N are equivalent.
- The difference in the probability of detection between live N and killed N ALO never exceeds 0.48.
- The difference in the probability of detection between live N and killed N ALO exceeds 0.40 over a range of 3 ACPLA
- The difference in the probability of detection between live N and killed N ALO exceeds 0.30 over a range of 5 ACPLA
- The difference in the probability of detection between live N and killed N ALO exceeds 0.20 over a range of 7 ACPLA
- The difference in the probability of detection between live N and killed N ALO exceeds 0.10 over a range of 10 ACPLA

- The difference in the probability of detection between live N and killed N ALO exceeds 0.05 over a range of 12.5 ACPLA

Clearly there is a relatively narrow range of concentration over which the simulant, killed N ALO produces JBPDS detection performance which is different from that produced by the agent, N.

Data from 504 field simulant challenges was used to construct an empirical distribution of challenge concentration. From this the probability of a field concentration was multiplied by the expected difference in performance between N and killed N ALO. These products were summed to produce the expected difference and are depicted in table 4-34. Clearly, over most of the concentration range of the field trials there is little or no difference in performance between live N and killed N ALO.

Table 4-34 Live N and Killed N ALO Expected Detection Difference

Difference in predicted detection performance between Agent and Simulant	**Fraction of Trials with Simulant Concentration that would produce the Difference**	**Expected Difference**
0.40-0.48	0.0119	0.034651
0.30-0.3999	0.0079	
0.20-0.2999	0.0020	
0.10-0.1999	0.0080	
0.05-0.0599	0.0139	
0.00-0.0499	0.9563	

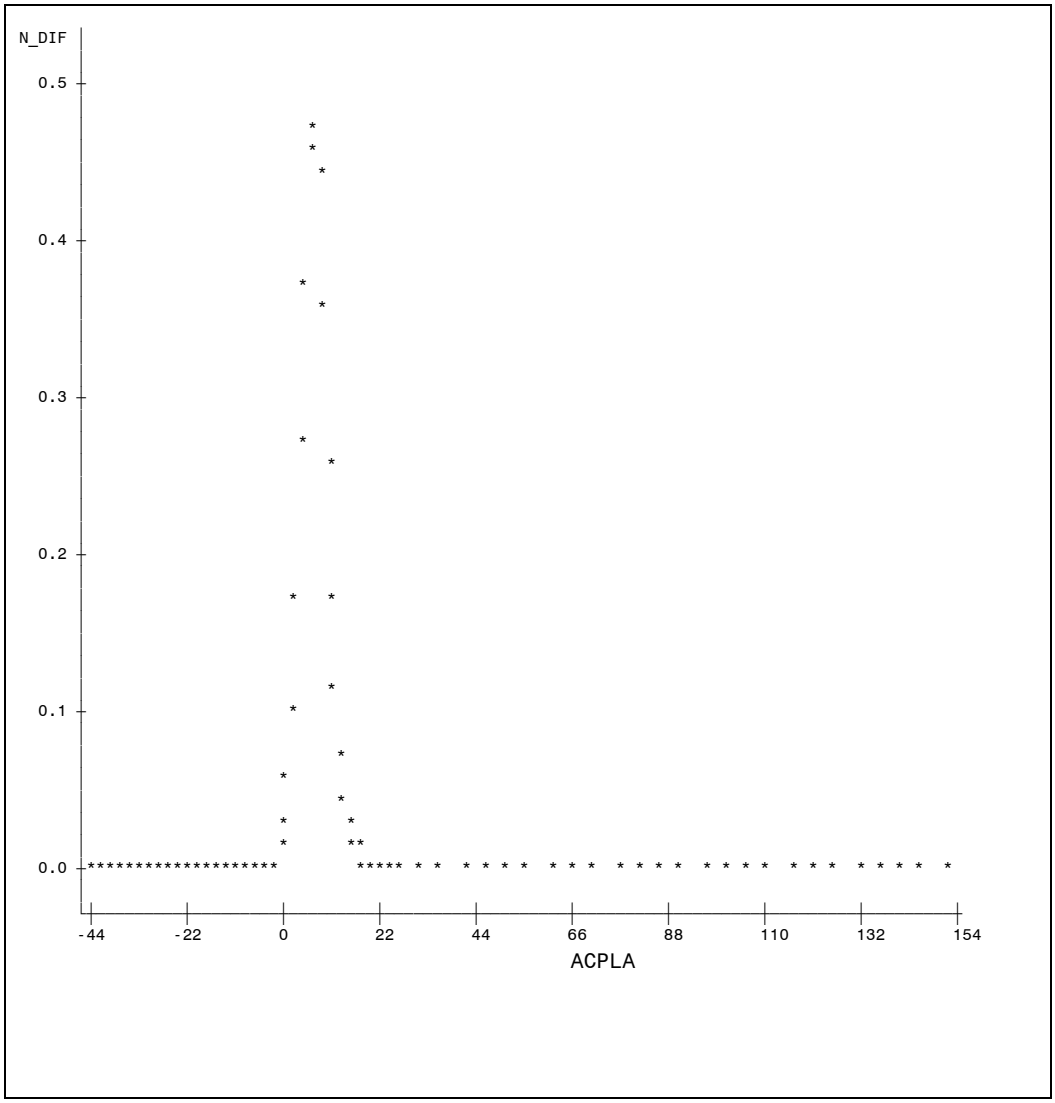


Figure 4-3 JBPDS Detection Performance. In this plot, N_DIF = P(Detect live N| Concentration)- P(Detect killed N ALO| Concentration). Concentration or ACPLA has been shifted to create an unclassified figure.

Detection Performance in the CAC with XR and Denatured XR

This section will provide an evaluation of hypothesis 3 for XR and denatured XR detection in the CAC. This section will provide insight to determine if JBPDS detection performance when challenged with XR is the same as when it is challenged with denatured XR. If not the same then determine the magnitude of that difference and how would it impact a field test. Since hypothesis 3 requires the use of actual biological warfare agent, the CAC is the only chamber where this hypothesis can be tested. The ultimate goal of this section is to determine if denatured XR is an acceptable simulant for XR.

The section conclusion is that denatured XR is an acceptable simulant for the detection of XR. JBPDS detection performance when challenged with XR biological warfare agent is only marginally statistically different from its detection performance when challenged with denatured XR simulant (p-value =0.0603). A meaningful difference in detection performance occurs over a range of 172 ACPLA.

XR CAC detection results are based on 61 XR challenges at various concentrations.

The JBPDS XR detection model fit statistics for the CAC are summarized in table 4-35. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-square of 0.81 indicates that there is predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 0.1525 with 6 degrees of freedom which produces a p-value of 0.99. The deviance goodness of fit statistic is 14.15 with 55 degrees of freedom and a p-value of 0.99. Neither goodness of fit tests are statistically significant which suggests that the model is not a bad fit.

Table 4-35 XR CAC Detection Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	70.050	25.698
SC	72.161	32.031
-2 Log L	68.050	19.698
R-Square 0.5474	Max-rescaled R-Square 0.8142	
Testing Global Null Hypothesis: All Coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	48.3516	2	<.0001
Score	19.0750	2	<.0001
Wald	4.1177	2	0.1276

The intercept and the natural log of concentration both contribute to this model (p-values of 0.0504 and 0.0490 respectively). As can be seen in table 4-36, there is weak statistical evidence that the detection performance of JBPDS when challenged with XR is different from when it is challenged with denatured XR. We conclude that in the CAC, JBPDS detection performance is somewhat different for XR and denatured XR (P-value=0.0603).

Table 4-36 XR CAC Detection Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	24.5463	3.8280	0.0504
Natural Log of Concentration	1	5.2175	3.8751	0.0490
Denatured Indicator	1	4.2058	3.5290	0.0603

The initial thought is that since the detection performance of JBPDS with XR is statistically different from its performance with denatured XR (P-value=0.0603), that denatured XR should not be used as a simulant for XR. However, before that conclusion

is reached and codified it is of interest to calculate the magnitude of the difference in JBPDS detection performance caused by these different challenges. If the difference is small enough, then even though it is statistically significant, denatured XR could still be an adequate simulant. The magnitude of that difference within the CAC will be examined in the remainder of this section. The magnitude of the difference in JBPDS detection performance in the CAC between XR and denatured XR will be estimated from the logistic regression model over different concentrations of challenge material.

The following observations are based on a comparison of predicted JBPDS detection performance against non-denatured XR in the CAC with the predicted JBPDS detection performance against denatured XR in the CAC (see Figure 4-4):

- The use of denatured XR as a stimulant for XR will result in either accurate estimations or underestimating JBPDS detection performance.
- At higher or lower concentrations the detection performance of JBPDS when challenged with denatured XR and non-denatured XR are equivalent.
- The difference in the probability of detection between XR and denatured XR never exceeds 0.96.
- The difference in the probability of detection between XR and denatured XR exceeds 0.90 over a range of 50 ACPLA
- The difference in the probability of detection between XR and denatured XR exceeds 0.80 over a range of 77 ACPLA
- The difference in the probability of detection between XR and denatured XR exceeds 0.70 over a range of 30.0 ACPLA

- The difference in the probability of detection between XR and denatured XR exceeds 0.60 over a range of 111 ACPLA
- The difference in the probability of detection between XR and denatured XR exceeds 0.50 over a range of 123 ACPLA
- The difference in the probability of detection between XR and denatured XR exceeds 0.40 over a range of 138 ACPLA
- The difference in the probability of detection between XR and denatured XR exceeds 0.30 over a range of 154 ACPLA
- The difference in the probability of detection between XR and denatured XR exceeds 0.20 over a range of 172 ACPLA
- The difference in the probability of detection between XR and denatured XR exceeds 0.10 over a range of 198 ACPLA
- The difference in the probability of detection between live NU and killed NU ALO exceeds 0.050 over a range of 228 ACPLA

Clearly there is a large range of concentration over which the simulant, denatured XR produces JBPDS detection performance which is different from that produced by the agent, XR.

Data from 504 field simulant challenges was used to construct an empirical distribution of challenge concentration. From this, the probability of a field concentration was multiplied by the expected difference in performance between XR and denatured XR. These products were summed to produce the expected difference and are depicted in table 4-37. Clearly, over most of the concentration range of the field trials there is little or no difference in performance between live XR and denatured XR. Furthermore, only

4.99% of the field challenges occurred in a range that would have produced a greater than 0.2 difference in detection performance between XR and denatured XR. Hence, denatured XR is an acceptable simulant for XR.

Table 4-37 XR and Denatured XR Expected Detection Difference

Difference in predicted detection performance between Agent and Simulant	**Fraction of Trials with Simulant Concentration that would produce the Difference**	**Expected Difference**
0.90-0.96	0.0100	0.0548925
0.80-0.8999	0.0040	
0.70-0.7999	0.0060	
0.60-0.6999	0.0020	
0.50-0.5999	0.0099	
0.40-0.4999	0.0020	
0.30-0.3999	0.0080	
0.20-0.2999	0.0080	
0.10-0.1999	0.0080	
0.05-0.0599	0.0099	
0.00-0.0499	0.9322	

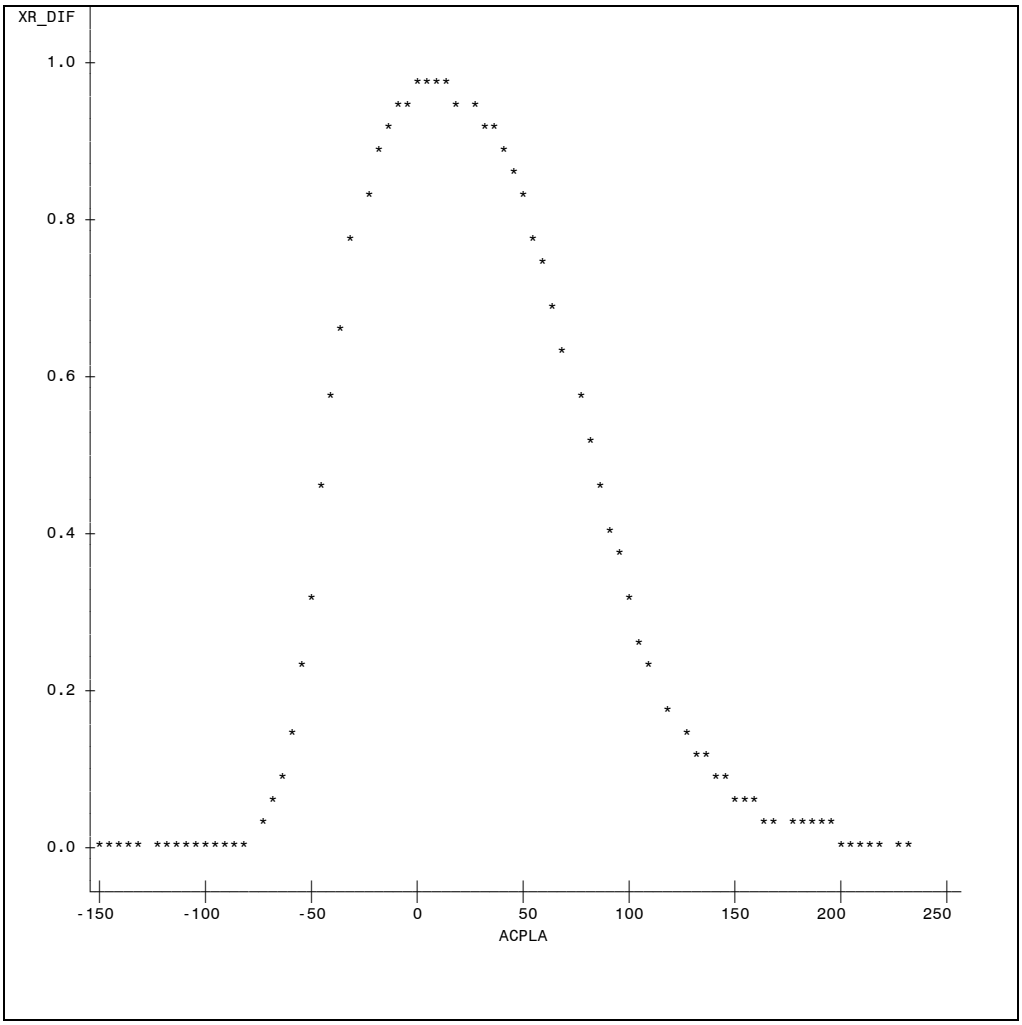


Figure 4-4 JBPDS Detection Performance. In this plot, XR_DIF = P(Detect XR| Concentration)- P(Detect denatured XR| Concentration). Concentration or ACPLA has been shifted to create an unclassified figure.

Identification Performance in the CAC with Killed and Live Agent (or Simulant)

Hypotheses 1 for identification will be evaluated in this section. Hypotheses 1 compares the identification performance of JBPDS when challenged with live agent or live simulant with identification performance of JBPDS when challenged with that same agent or simulant after it has been killed. The purpose of this hypothesis is twofold. First, if performance with the killed ALO is different from performance with live agent, it provides a scientific explanation as to if the killing process contributes to the difference in performance. Second, if the killing process resulted in the difference in performance between live agent and its corresponding killed ALO, it provides information that could guide changes in the killing process that may improve the ALO simulant.

The following summary of results is developed in this section:

- Based on the inability for JBPDS to identify killed LE, Killed N ALO, washed N ALO, and NU ALO at concentrations in which the corresponding live agent or simulant is readily identified, the null hypothesis in hypothesis 1 is rejected for those pairs of agent or simulant and their corresponding killed agent or simulant. That is to say, for LE, N ALO, washed N ALO, and NU ALO JBPDS detection performance when challenged with live agent (or simulant) is considerably different from its performance when challenged with the corresponding killed agent (or simulant).
- The null hypothesis in hypothesis 1 is not rejected for the follow pairs of agent or simulant and their corresponding killed agent or simulant: live LE ALO and killed LE ALO (p-value=0.9432) and live N and killed N (p-

value=0.9420). That is to say for these agents and simulants there is no evidence to suggest that JBPDS detection performance when challenged with live agent or simulant is different from its performance when challenged with the corresponding killed agent.

LE CAC identification results are based on 37 LE challenges at various concentrations.

The LE model which is depicted in tables 4-38 and 4-39 includes an intercept, the natural log of the concentration, and an indicator parameter for living or killed LE. Regrettably, there was quasi-complete separation of data points in this model and hence, the maximum likelihood estimate may not exist. This model is not suited for analysis with logistic regression.

Table 4-38 LE CAC Identification Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	34.800	17.493
SC	36.411	22.326
-2 Log L	32.800	11.493
R-Square 0.4378	Max-rescaled R-Square 0.7446	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF Pr > ChiSq
Likelihood Ratio	21.3061	2 <.0001
Score	20.0221	2 <.0001
Wald	6.6469	2 0.0360

Table 4-39 LE CAC Identification Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	83.4039	0.0508	0.8218
Natural Log of Concentration	1	1.0542	6.6417	0.0100
Live or Killed Indicator	1	83.2669	0.0021	0.9636

The inability for JBPDS to identify killed LE at concentrations in which the JBPDS can readily identify live LE is the cause of quasi-complete separation of data points. Hence, it can be concluded that the identification performance of JBPDS is different between challenges of live and killed LE.

The data set on LE identification in the CAC is not suitable to support a comparison of JBPDS identification performance differences between live and killed LE.

LE ALO CAC identification results are based on 48 LE ALO challenges at various concentrations.

The JBPDS LE identification model fit statistics for the CAC are summarized in table 4-40. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-square of 0.69 indicates that there is predictive value in this model, but not as much as some of the others.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 19.17 with 8 degrees of freedom which produces a p-value of 0.0139 which suggests that this is not the best model fit. However, the deviance goodness of fit statistic is 27.91 with 44 degrees of freedom and a p-value of 0.97 which suggests that this model is not a bad fit.

Table 4-40 LE ALO CAC Identification Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	63.105	33.909
SC	64.977	39.523
-2 Log L	61.105	27.909
R-Square 0.4992	Max-rescaled R-Square 0.6933	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	33.1961	2	<.0001
Score	26.5847	2	<.0001
Wald	11.3312	2	0.0035

The intercept and the natural log of concentration both contribute to this model (p-values of 0.0020 and 0.0014 respectively). As can be seen in table 4-41, there is no evidence that the identification performance of JBPDS when challenged with live LE ALO is different from when it is challenged with dead LE ALO. We conclude that in the CAC, JBPDS identification performance is the same with killed and live LE ALO (P-value=0.9432).

Table 4-41 LE ALO CAC Identification Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	3.8078	9.5325	0.0020
Natural Log of Concentration	1	0.5357	10.1480	0.0014
LIVE	1	0.5136	0.0051	0.9432

N CAC identification results are based on 32 N challenges at various concentrations.

The JBPDS N identification model fit statistics for the CAC are summarized in table 4-42. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-square of 0.82 indicates that there is predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 4.34 with 7 degrees of freedom which produces a p-value of 0.7385. The deviance goodness of fit statistic is 11.58 with 28 degrees of freedom and a p-value of 0.99. Neither goodness of fit tests are statistically significant which suggests that the model is not a bad fit.

Table 4-42 N CAC Identification Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	41.750	17.584
SC	43.215	21.981
-2 Log L	39.750	11.584
R-Square 0.5853	Max-rescaled R-Square 0.8229	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	28.1654	2	<.0001
Score	19.0559	2	<.0001
Wald	3.1446	2	0.2076

The natural log of concentration contributes to this model (p-value=0.0764). As can be seen in table 4-43, there is no evidence that the identification performance of JBPDS when challenged with live N is different from when it is challenged with dead N. We conclude that in the CAC, JBPDS identification performance is the same with killed and live N (P-value=0.9420).

Table 4-43 N CAC Identification Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	99.9585	0.1531	0.6956
Natural Log of Concentration	1	3.5278	3.1402	0.0764
LIVE	1	98.1697	0.0053	0.9420

N ALO CAC identification results are based on 24 N ALO challenges at various concentrations.

The N ALO model which is depicted in tables 4-44 and 4-45 includes an intercept, the natural log of the concentration, and an indicator parameter for living or killed N ALO. Regrettably, there was quasi-complete separation of data points in this model and hence, the maximum likelihood estimate may not exist. This model is not suited for analysis with logistic regression.

Table 4-44 N ALO CAC Identification Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	10.314	6.079
SC	11.492	9.613
-2 Log L	8.314	0.079
R-Square 0.2904	Max-rescaled R-Square 0.9920	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	8.2349	2	0.0163
Score	3.2462	2	0.1973
Wald	0.5956	2	0.7425

Table 4-45 N ALO CAC Identification Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	764.1	0.5719	0.4495
Natural Log of Concentration	1	153.0	0.5644	0.4525
Live or Killed Indicator	1	61.2729	0.5922	0.4416

The inability for JBPDS to identify killed N ALO at concentrations in which the JBPDS can readily identify live N ALO is the cause of quasi-complete separation of data points. Hence, it can be concluded that the identification performance of JBPDS is different between challenges of live and killed N ALO.

Washed N ALO CAC identification results are based on 24 N ALO challenges at various concentrations.

The washed N ALO model which is depicted in tables 4-46 and 4-47 includes an intercept, the natural log of the concentration, and an indicator parameter for living or killed N ALO. Regrettably, there was quasi-complete separation of data points in this model and hence, the maximum likelihood estimate may not exist. This model is not suited for analysis with logistic regression.

Table 4-46 Washed N ALO CAC Identification Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	15.768	13.691
SC	16.946	17.225
-2 Log L	13.768	7.691
R-Square 0.2237	Max-rescaled R-Square 0.5124	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	6.0770	2	0.0479
Score	5.3807	2	0.0679
Wald	0.6997	2	0.7048

Table 4-47 Washed N ALO CAC Identification Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	106.4	0.0577	0.8102
Natural Log of Concentration	1	4.5961	0.6964	0.4040
Live or Killed Indicator	1	103.9	0.0020	0.9642

The inability for JBPDS to identify killed washed N ALO at concentrations in which the JBPDS can readily identify live washed N ALO is the cause of quasi-complete separation of data points. Hence, it can be concluded that the identification performance of JBPDS is different between challenges of live and killed washed N ALO.

NU ALO CAC identification results are based on 42 NU ALO challenges at various concentrations.

The NU ALO model which is depicted in tables 4-48 and 4-49 includes an intercept, the natural log of the concentration, and an indicator parameter for living or killed NU ALO. Regrettably, there was quasi-complete separation of data points in this

model and hence, the maximum likelihood estimate may not exist. This model is not suited for analysis with logistic regression.

Table 4-48 NU ALO CAC Identification Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	42.901	21.118
SC	44.638	26.331
-2 Log L	40.901	15.118
R-Square 0.4588	Max-rescaled R-Square 0.7371	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	25.7828	2	<.0001
Score	24.4050	2	<.0001
Wald	0.1655	2	0.9206

Table 4-49 NU ALO CAC Identification Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	97.6863	0.0714	0.7893
Natural Log of Concentration	1	7.1810	0.1594	0.6897
Live or Killed Indicator	1	84.9956	0.0000	0.9997

The inability for JBPDS to identify killed NU ALO at concentrations in which the JBPDS can readily identify live NU ALO is the cause of quasi-complete separation of data points. Hence, it can be concluded that the identification performance of JBPDS is different between challenges of live and killed NU ALO.

Identification Performance in the CAC with Live Agent and Live ALO

Hypotheses 2 for identification will be evaluated in this section. Hypotheses 2 compares the identification performance of JBPDS when challenged with live agent to identification performance of JBPDS when challenged with the corresponding killed ALO. The purpose of this hypothesis is twofold. First, if performance with the killed ALO is different from performance with live agent, then results from this hypothesis provides a scientific explanation as to if a component of this difference is caused by the basic difference between live agent and live ALO. Second, if in fact the identification performance is different between live ALO and live agent, then in future efforts simulant properties could be improved by choosing a different ALO.

The following summary of results is developed in this section:

- The null hypothesis in hypothesis 2 is rejected for live LE compared to live LE ALO (p-value=0.0300). That is to say, for LE JBPDS identification performance when challenged with live agent is statistically different from its performance when challenged with the corresponding ALO.
- The null hypothesis in hypothesis 2 is not rejected for live N and live N ALO (p-value=0.9490). That is to say for this live agents and live ALO there is no evidence to suggest that JBPDS identification performance when challenged with live agent or is different from its performance when challenged with the corresponding live ALO.

Live LE and live LE ALO CAC identification results are based on 48 challenges at various concentrations.

The JBPDS Live LE and live LE ALO identification model fit statistics for the CAC are summarized in table 4-50. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-square of 0.85 indicates that there is predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 2.0041 with 8 degrees of freedom which produces a p-value of 0.99. The deviance goodness of fit statistic is 13.26 with 44 degrees of freedom and a p-value of 0.99. Neither goodness of fit tests are statistically significant which suggests that the model is not a bad fit.

Table 4-50 Live LE versus Live LE ALO Identification CAC Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	55.984	19.257
SC	57.855	24.870
-2 Log L	53.984	13.257
R-Square 0.5719	Max-rescaled R-Square 0.8470	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	40.7277	2	<.0001
Score	28.9827	2	<.0001
Wald	11.0773	2	0.0039

The intercept and the natural log of concentration both contribute to this model (p-values of 0.0009 and 0.0009 respectively). As can be seen in table 4-51, there is evidence that the detection performance of JBPDS when challenged with Live LE is different from when it is challenged with Live LE ALO. We conclude that in the CAC, JBPDS detection performance is not the same with live LE as it is with the corresponding live LE ALO (P-value=0.0300).

Table 4-51 Live LE versus Live LE ALO Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	6.4958	10.9629	0.0009
Natural Log of Concentration	1	1.0480	11.0697	0.0009
LE or LE ALO Indicator	1	1.0745	4.7107	0.0300

Live N and live N ALO CAC identification results are based on 32 challenges at various concentrations.

The JBPDS Live N and live N ALO identification model fit statistics for the CAC are summarized in table 4-52. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-square of 0.82 indicates that there is predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 4.3517 with 7 degrees of freedom which produces a p-value of 0.74. The deviance goodness of fit statistic is 11.58 with 28 degrees of freedom and a p-value of 0.99. Neither goodness of fit tests are statistically significant which suggests that the model is not a bad fit.

Table 4-52 Live N versus Live N ALO Identification CAC Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	41.750	17.584
SC	43.215	21.981
-2 Log L	39.750	11.584
R-Square 0.5853	Max-rescaled R-Square 0.8229	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	28.1654	2	<.0001
Score	20.5405	2	<.0001
Wald	3.1442	2	0.2076

The natural log of concentration contribute to this model (p-values=0.0764). As can be seen in table 4-53, there is no evidence that the detection performance of JBPDS when challenged with Live N is different from when it is challenged with Live N ALO. We conclude that in the CAC, JBPDS detection performance is same with live N as it is with the corresponding live N ALO (P-value=0.9490).

Table 4-53 Live N versus Live N ALO Identification CAC Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	103.3	0.1388	0.7095
Natural Log of Concentration	1	3.5277	3.1402	0.0764
N or N ALO Indicator	1	101.6	0.0041	0.9490

Live NU and live NU ALO CAC identification results are based on 41 challenges at various concentrations. Regrettably, because of test limitations sufficient data was not generated in all groups to analyze. No statistics could be generated.

Identification Performance in the CAC with Live LE and Killed LE ALO

This section will provide an evaluation of hypothesis 3 for live LE and killed LE ALO identification in the CAC. This section will provide insight to determine if JBPDS identification performance when challenged with live LE is the same as when it is challenged with killed LE ALO. If not the same then determine the magnitude of that difference and how would it impact a field test. Since hypothesis 3 requires the use of actual biological warfare agent, the CAC is the only chamber where this hypothesis can be tested. The ultimate goal of this section is to determine if killed LE ALO is an acceptable simulant for live LE.

The section conclusion is that killed LE ALO is an acceptable simulant for LE biological warfare agent. There was no statistical difference in JBPDS identification performance when challenged with killed Le ALO from JBPDS identification performance when challenged with Le biological warfare agent (p-value=0.1562).

Live LE and Killed LE ALO CAC identification results are based on 50 challenges at various concentrations.

The JBPDS live LE and Killed LE ALO identification model fit statistics for the CAC are summarized in table 4-54. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-square of 0.59 indicates that there is predictive value in this model, but not as much as some of the others.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 11.6213 with 8 degrees of freedom which produces a p-value of 0.17. The deviance goodness of fit statistic is 35.07 with 45 degrees of freedom and a p-value of 0.86. Neither goodness of fit tests are statistically significant which suggests that the model is not a bad fit.

Table 4-54 Live LE versus Killed LE ALO CAC Identification Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	64.687	41.073
SC	66.599	46.809
-2 Log L	62.687	35.073
R-Square 0.4244	Max-rescaled R-Square 0.5939	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	27.6137	2	<.0001
Score	23.6857	2	<.0001
Wald	13.6306	2	0.0011

The intercept and the natural log of concentration both contribute to this model (p-values of 0.0009 and 0.0008 respectively). As can be seen in table 4-55, there is little if any evidence that the detection performance of JBPDS when challenged with live LE is different from when it is challenged with killed LE ALO. We conclude that JBPDS performance is the same with killed LE ALO or live LE agent.

Table 4-55 Live LE versus Killed LE ALO Analysis of CAC Identification Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	2.8771	10.9568	0.0009
Natural Log of Concentration	1	0.4556	11.1375	0.0008
Live LE or Killed LE ALO Indicator	1	0.6385	2.0104	0.1562

As seen in table 5-56, the process of killing has a tremendous effect on identification performance of killed LE but not killed LE ALO. It is quite interesting that there is statistical significance in performance of live LE when compared to live LE ALO (p-value=0.0300) but no statistical difference in performance when live LE is compared to killed LE ALO (p-value=0.1562).

Table 4-56 LE Agent and Simulant Identification Summery of P-values

Comparison	Source	P-value
Live LE versus Killed LE	Narrative around Table 4-39	Unable to identify killed LE
Live LE ALO versus Killed LE ALO	Table 4-41	0.9432
Live LE versus Live LE ALO	Table 4-51	0.0300
Live LE versus Killed LE ALO	Table 4-55	0.1562

The magnitude of that difference in JBPDS identification performance between killed LE ALO and live LE will be examined in the remainder of this section.

The magnitude of the difference in JBPDS identification performance between killed LE ALO and live LE agent will be estimated from the logistic regression model over different concentrations of challenge material.

The following observations are based on a comparison of predicted JBPDS identification performance against live LE with the JBPDS identification performance against killed LE ALO (see Figure 4-5):

- The use of killed LE ALO as a stimulant for live LE will result in either accurate estimations or underestimating JBPDS identification performance.
- At lower concentrations the detection performance of JBPDS when challenged with killed LE ALO and live LE are equivalent. At all reasonable challenge concentrations, at higher concentrations killed LE ALO will result underestimating JBPDS identification performance
- The difference in the probability of detection between killed LE ALO and live LE agent never exceeds 0.23.
- The difference in the probability of detection between killed LE ALO and live LE agent exceeds 0.20 over a range of 640 ACPLA
- The difference in the probability of detection between killed LE ALO and live LE agent exceeds 0.10 over a range of 2,300 ACPLA
- The difference in the probability of detection between killed LE ALO and live LE agent exceeds 0.050 over a range of 4,290 ACPLA

Clearly there is a large range of concentration over which the simulant killed LE ALO will underestimate JBPDS identification performance against the live agent LE. However, the underestimation is never more that 0.23 is often less than 0.10. It is also noteworthy to emphasize that that difference is not statistically different.

Data from 504 field simulant challenges was used to construct an empirical distribution of challenge concentration. From this, the probability of a field concentration was multiplied by the expected difference in performance between live LE and killed LE ALO. These products were summed to produce the expected difference and are depicted in table 4-57. Clearly, over most of the concentration range of the field trials there is little or no difference in performance between live LE and killed LE ALO.

Table 4-57 LE and Denatured LE Expected Identification Difference

Difference in predicted detection performance between Agent and Simulant	**Fraction of Trials with Simulant Concentration that would produce the Difference**	**Expected Difference**
0.20-0.23	0.0280	0.0439825
0.10-0.1999	0.0837	
0.05-0.0599	0.0640	
0.00-0.0499	0.8243	

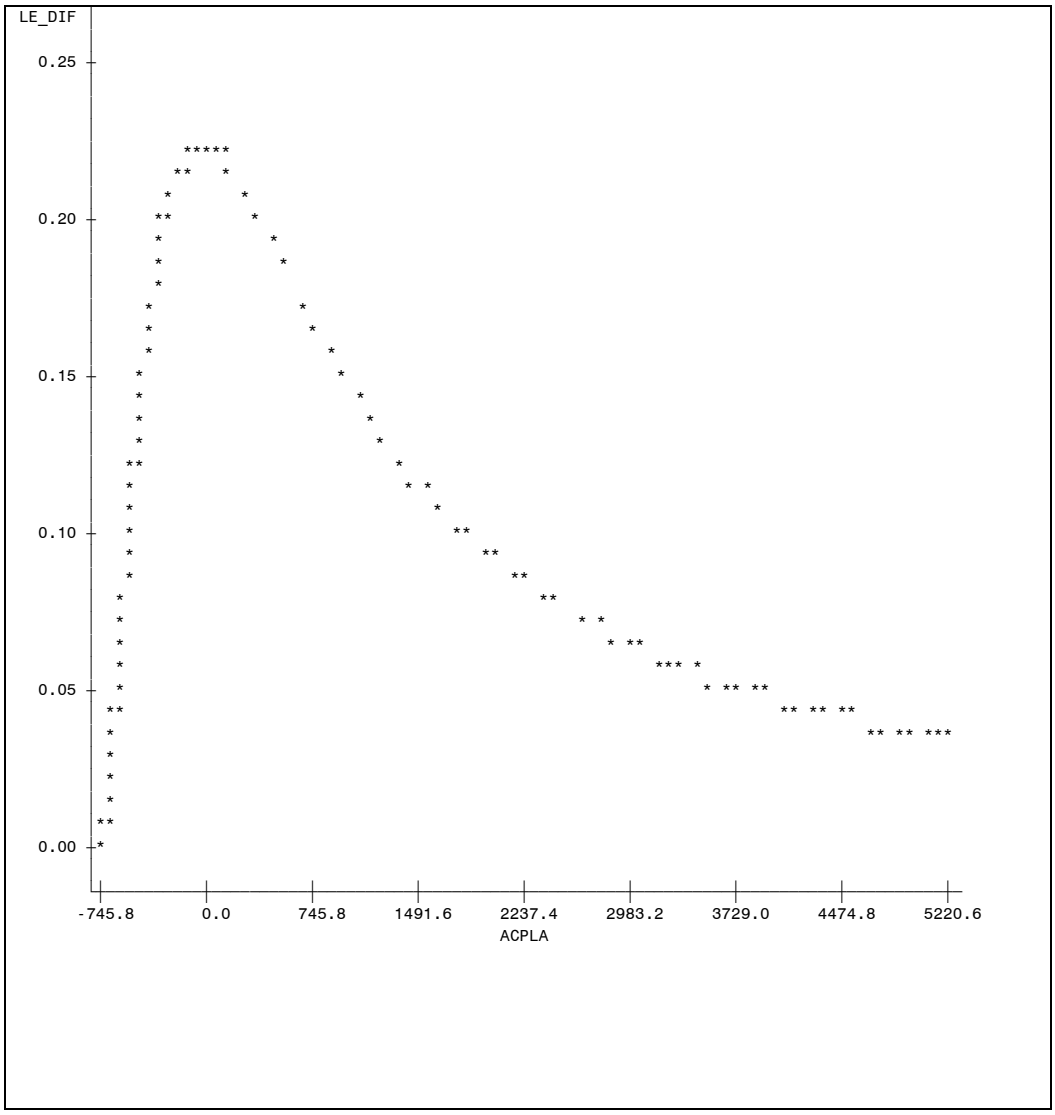


Figure 4-5 JBPDS LE Identification Performance. In this plot, LE_DIF = P(Identify LE| Concentration)- P(Identify denatured LE| Concentration). Concentration or ACPLA has been shifted to create an unclassified figure.

Identification Performance in the CAC with Live NU and Killed NU ALO

This section will provide an evaluation of hypothesis 3 for live NU and killed NU ALO identification in the CAC. This section will provide insight to determine if JBPDS identification performance when challenged with live NU is the same as when it is

challenged with killed NU ALO. If not the same then determine the magnitude of that difference and how would it impact a field test. Since hypothesis 3 requires the use of actual biological warfare agent, the CAC is the only chamber where this hypothesis can be tested. The ultimate goal of this section is to determine if killed NU ALO is an acceptable simulant for live NU.

The section conclusion is that Killed Nu ALO is not an acceptable simulant for Nu biological warfare agent. At concentrations in which killed NU ALO was readily identified, JBPDS failed to identify any Nu biological warfare agent aerosol challenges.

Live NU and Killed NU ALO CAC identification results are based on 59 challenges at various concentrations.

The Live NU and Killed NU ALO model which is depicted in tables 4-58 and 4-59 includes an intercept, the natural log of the concentration, and an indicator parameter for living NU or killed NU ALO. Regrettably, there was quasi-complete separation of data points in this model and hence, the maximum likelihood estimate may not exist. This model is not suited for analysis with logistic regression.

Table 4-58 Live NU versus Killed NU ALO CAC Identification Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	48.832	21.118
SC	50.910	27.351
-2 Log L	46.832	15.118
R-Square 0.4158	Max-rescaled R-Square 0.7590	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	31.7142	2	<.0001
Score	31.5330	2	<.0001
Wald	0.1657	2	0.9205

Table 4-59 Live NU versus Killed NU ALO Analysis of CAC Identification Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	105.1	0.0917	0.7620
Natural Log of Concentration	1	7.1811	0.1594	0.6897
Live NU or Killed NU ALO Indicator	1	82.6809	0.0049	0.9443

The cause of the quasi-complete separation data points is that at concentrations in which killed NU ALO is readily identified, live NU is not. This order of ease of identification is counterintuitive. This leads to the conclusions that:

- The identification performance when challenged with live NU is different from the identification performance when challenged with killed NU ALO.
- That difference would be great and result in overestimating identification performance based on killed NU ALO field identification performance.
- Killed NU ALO is not an acceptable simulant for Nu identification performance.

Identification Performance in the CAC with Live N and Killed N ALO

This section will provide an evaluation of hypothesis 3 for live N and killed N ALO identification in the CAC. This section will provide insight to determine if JBPDS identification performance when challenged with live N is the same as when it is challenged with killed N ALO. If not the same, then determine the magnitude of that difference and how it would impact a field test. Since hypothesis 3 requires the use of actual biological warfare agent, the CAC is the only chamber where this hypothesis can be tested. The ultimate goal of this section is to determine if killed N ALO is an acceptable simulant for live N.

The section conclusion is that Killed N ALO is not an acceptable simulant for N biological warfare agent. At concentrations in which N biological warfare agent was readily identified, JBPDS failed to identify any killed N ALO aerosol challenges.

Live N and Killed N ALO CAC identification results are based on 32 challenges at various concentrations.

The Live N and Killed N ALO model which is depicted in tables 4-60 and 4-61 includes an intercept, the natural log of the concentration, and an indicator parameter for living N or killed N ALO. Regrettably, there was quasi-complete separation of data points in this model and hence, the maximum likelihood estimate may not exist. This model is not suited for analysis with logistic regression.

Table 4-60 Live N versus Killed N ALO CAC Identification Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	43.183	21.328
SC	44.649	25.726
-2 Log L	41.183	15.328
R-Square 0.5542	Max-rescaled R-Square 0.7656	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	25.8551	2	<.0001
Score	18.9756	2	<.0001
Wald	5.6616	2	0.0590

Table 4-61 Live N versus Killed N ALO Analysis of CAC Identification Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	20.8793	3.3164	0.0686
Natural Log of Concentration	1	4.2654	3.2588	0.0710
Live N or Killed N ALO Indicator	1	1.5934	0.9164	0.3384

The cause of the quasi-complete separation data points is that at concentrations in which live N is readily identified, killed N ALO is not. This leads to the conclusions that:

- The identification performance when challenged with live N is different from the identification performance when challenged with killed N ALO.
- That difference would be great and result in underestimating identification performance based on killed N ALO field identification performance.
- Killed N ALO is not an acceptable simulant for N identification performance.

Identification Performance in the CAC with XR and Denatured XR

This section will provide an evaluation of hypothesis 3 for XR and denatured XR identification in the CAC. This section will provide insight to determine if JBPDS identification performance when challenged with XR is the same as when it is challenged with denatured XR. If not the same then determine the magnitude of that difference and how would it impact a field test. Since hypothesis 3 requires the use of actual biological warfare agent, the CAC is the only chamber where this hypothesis can be tested. The ultimate goal of this section is to determine if denatured XR is an acceptable simulant for XR.

The section conclusion is that denatured XR is an acceptable simulant for the identification of XR biological warfare agent. JBPDS identification performance when challenged with XR biological warfare agent is statistically different from its identification performance when challenged with denatured XR simulant (p-value =0.0294). A meaningful difference in detection performance occurs over a range of 7,150 ACPLA, which is quite large. However, based on the location of that range that results in different performance, few field trials would be expected to be on that range.

XR CAC identification results are based on 46 XR challenges at various concentrations.

The JBPDS XR identification model fit statistics for the CAC are summarized in table 4-62. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-square of 0.60 indicates that there is predictive value in this model, but not as much as some of the others.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 3.5285 with 6 degrees of freedom which produces a p-value of 0.74. The deviance goodness of fit statistic is 29.89 with 43 degrees of freedom and a p-value of 0.93. Neither goodness of fit tests are statistically significant which suggests that the model is not a bad fit.

Table 4-62 XR CAC Identification Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	56.777	35.893
SC	58.605	41.379
-2 Log L	54.777	29.893
R-Square 0.4178	Max-rescaled R-Square 0.6003	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	24.8837	2	<.0001
Score	13.3797	2	0.0012
Wald	5.8534	2	0.0536

The intercept and the natural log of concentration both contribute statistically to this model (p-values of 0.0202 and 0.0201 respectively). As can be seen in table 4-63, there is evidence that the identification performance of JBPDS when challenged with live XR is different from when it is challenged with dead XR. We conclude that in the CAC, JBPDS identification performance is different for killed and live XR (P-value=0.0294).

Table 4-63 XR CAC Identification Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	19.2380	5.3945	0.0202
Natural Log of Concentration	1	2.4428	5.4042	0.0201
LIVE	1	2.6615	4.7448	0.0294

The initial thought is that since the identification performance of JBPDS with XR is statistically different from its performance with denatured XR (P-value=0.0294), that denatured XR should not be used as a simulant for XR. However, before that conclusion is reached and codified it is of interest to calculate the magnitude of the difference in JBPDS identification performance caused by these different challenges. If the difference is small enough, then even though it is statistically significant, denatured XR could still be an adequate simulant. The magnitude of that difference will be examined in the remainder of this section.

The magnitude of the difference in JBPDS identification performance between XR and denatured XR will be estimated from the logistic regression model over different concentrations of challenge material.

The following observations are based on a comparison of predicted JBPDS identification performance against non-denatured XR with the predicted JBPDS identification performance against denatured (see Figure 4-6):

- The use of denatured XR as a stimulant for XR will result in either accurate estimations or underestimating JBPDS identification performance.
- At higher or lower concentrations the identification performance of JBPDS when challenged with denatured XR and non-denatured XR are equivalent. However, at most high concentrations denatured XR will underestimate performance.
- The difference in the probability of identification between XR and denatured XR never exceeds 0.90.

- The difference in the probability of identification between XR and denatured XR exceeds 0.80 over a range of 2,100 ACPLA
- The difference in the probability of identification between XR and denatured XR exceeds 0.70 over a range of 3,100 ACPLA
- The difference in the probability of identification between XR and denatured XR exceeds 0.60 over a range of 3,900 ACPLA
- The difference in the probability of identification between XR and denatured XR exceeds 0.50 over a range of 4,550 ACPLA
- The difference in the probability of identification between XR and denatured XR exceeds 0.40 over a range of 5,300 ACPLA
- The difference in the probability of identification between XR and denatured XR exceeds 0.30 over a range of 6,150 ACPLA
- The difference in the probability of identification between XR and denatured XR exceeds 0.20 over a range of 7,150 ACPLA
- The difference in the probability of identification between XR and denatured XR exceeds 0.10 over a range of 8,700 ACPLA
- The difference in the probability of identification between XR and denatured XR exceeds 0.050 over a range of 8,700 ACPLA

Clearly there is a large range of concentration over which the simulant, denatured XR produces JBPDS identification performance which is different from that produced by the agent, XR.

Data from 504 field simulant challenges was used to construct an empirical distribution of challenge concentration. From this, the probability of a field concentration

was multiplied by the expected difference in performance between XR and denatured XR. These products were summed to produce the expected difference and are depicted in table 4-64. Clearly, over most of the concentration range of the field trials there is little or no difference in performance between live XR and denatured XR. Furthermore, only 4.99% of the field trials were at a concentration that would have resulted in greater than 0.2 difference between predicted identification performance when challenged with XR as opposed to denatured XR. Based in this finding, denatured XR is an acceptable identification simulant for XR in field trials.

Table 4-64 XR and Denatured XR Expected Identification Difference

Difference in predicted detection performance between Agent and Simulant	**Fraction of Trials with Simulant Concentration that would produce the Difference**	**Expected Difference**
0.90	0	0.0524675
0.80-0.8999	0.0120	
0.70-0.7999	0.0040	
0.60-0.6999	0.0060	
0.50-0.5999	0.0040	
0.40-0.4999	0.0060	
0.30-0.3999	0.0079	
0.20-0.2999	0.0100	
0.10-0.1999	0.0100	
0.05-0.0599	0.0040	
0.00-0.0499	0.9361	

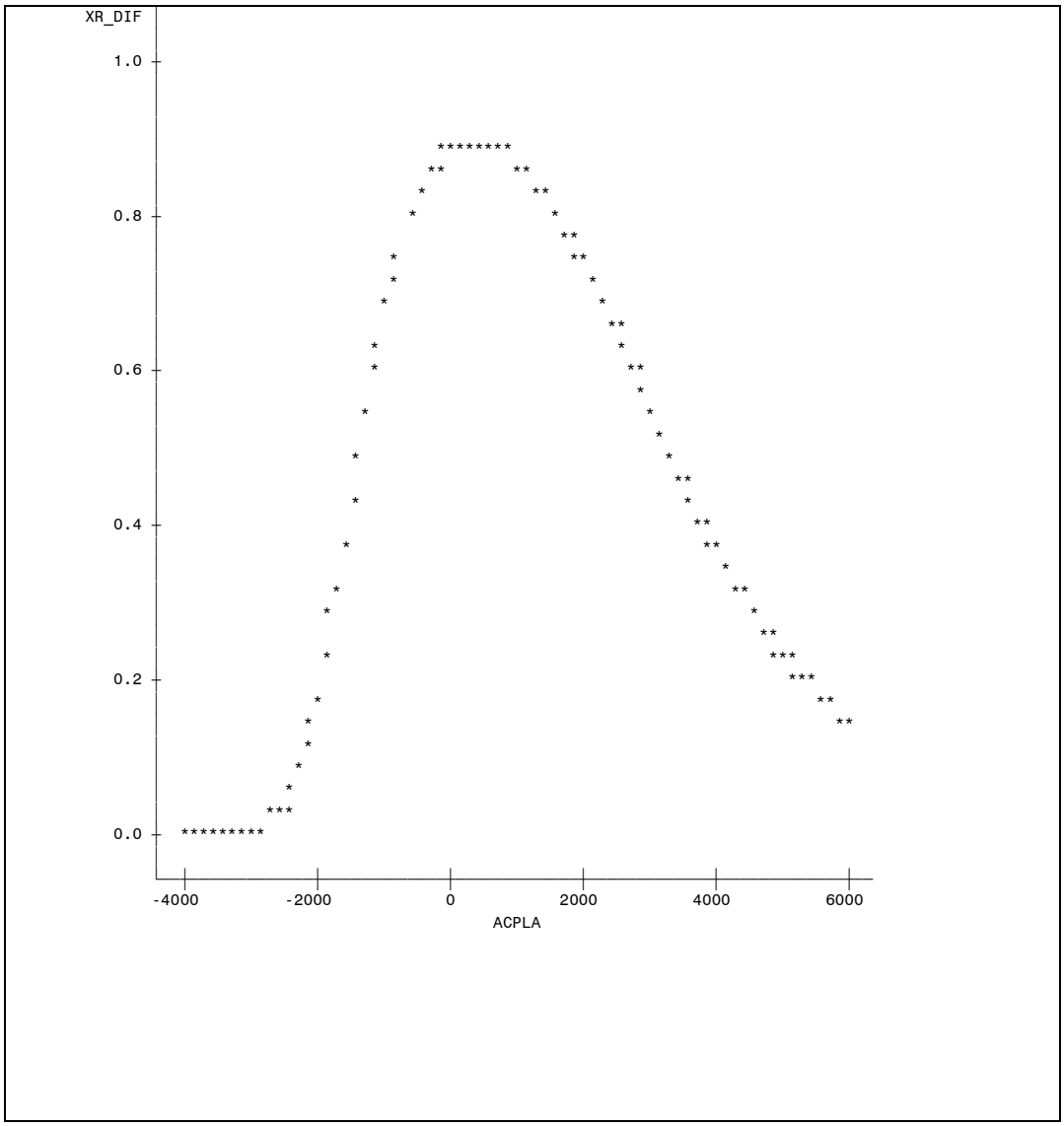


Figure 4-6 JBPDS Identification Performance. In this plot,
XR_DIF = P(Identify XR| Concentration)- P(Identify denatured XR| Concentration).
Concentration or ACPLA has been shifted to create an unclassified figure.

Detection Performance in the ASEC with Killed and Live Agent

Hypotheses 1 for detection in the ASEC will be evaluated in this section. Hypotheses 1 compares the detection performance of JBPDS when challenged with live agent or live simulant with detection performance of JBPDS when challenged with that same agent or simulant after it has been killed. BG is the only live organism to be released in the ASEC. Hence, this section will only compare live BG to Killed BG.

The following summary of results is developed in this section. The null hypothesis in hypothesis 1 is not rejected for the live BG and killed BG (p-value=0.1336). That is to say there is no evidence to suggest that JBPDS detection performance when challenged with live BG is different from its performance when challenged with killed BG.

BG ASEC detection results are based on 98 BG challenges at various concentrations of which 58 were detected and 40 were not detected.

The JBPDS BG detection model fit statistics for the ASEC are summarized in table 4-65. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-Square of 0.66 indicates that there is predictive value in this model, but not as much as some of the others.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 25.27 with 7 degrees of freedom which produces a p-value of 0.0007 which suggests that the model is not a good fit. The deviance goodness of fit statistic is 57.80 with 82 degrees of freedom and a p-value of 0.9804 which suggests that the model is not a bad fit.

Table 4-65 BG ASEC Detection Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	134.532	75.174
SC	137.117	85.514
-2 Log L	132.532	67.174
R-Square 0.4867	Max-rescaled R-Square 0.6565	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF Pr > ChiSq
Likelihood Ratio	65.3582	3 <.0001
Score	36.1725	3 <.0001
Wald	19.3791	3 0.0002

The intercept, the natural log of concentration, and the JBPDS indicator parameter all contribute to this model (p-values of <.0001, <.0001, and 0.0830 respectively). As can be seen in table 4-66, there is little if any evidence that the detection performance of JBPDS when challenged with live BG is different from when it is challenged with dead BG. We conclude that in the ASEC, JBPDS detection performance is the same with killed and live BG (P-value=0.1336).

Table 4-66 BG ASEC Detection Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	-15.2397	3.5622	18.3031	<.0001
Natural Log of Concentration	1	4.5450	1.0502	18.7299	<.0001
JBPDS Indicator	1	0.5387	0.3107	3.0059	0.0830
Live or Killed BG Indicator	1	-0.5245	0.3496	2.2506	0.1336

Identification Performance in the ASEC with Killed and Live Agent

Hypotheses 1 for identification in the ASEC will be evaluated in this section. Hypotheses 1 compares the identification performance of JBPDS when challenged with

live agent or live simulant with detection performance of JBPDS when challenged with that same agent or simulant after it has been killed. BG is the only live organism to be released in the ASEC. Hence, this section will only compare live BG to Killed BG.

The following summary of results is developed in this section. Because of the narrow distribution of concentration, especially at the lower concentrations were identification tends to fail, no inferences are reached on hypothesis 1 for live and killed BG in the ABT.

BG ASEC identification results are based on 58 BG challenges at various concentrations of which 52 were identified and 6 were not identified.

The BG model which is depicted in tables 4-67 and 4-68 includes an intercept, the natural log of the concentration, indicator parameters to identify the JBPDS, and an indicator parameter for living or dead BG. Regrettably, there was quasi-complete separation of data points in this model and hence, the maximum likelihood estimate may not exist. This model is not suited for analysis with logistic regression.

Table 4-67 BG ASEC Identification Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	40.581	31.751
SC	42.641	39.993
-2 Log L	38.581	23.751
R-Square 0.2256	Max-rescaled R-Square 0.4644	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	14.8297	3	0.0020
Score	12.5681	3	0.0057
Wald	1.1979	3	0.7535

Table 4-68 BG ASEC Identification Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	12.3931	96.5941	0.0165	0.8979
Natural Log of Concentration	1	-1.0346	2.1085	0.2408	0.6237
JBPDS Indicator	1	-0.6830	0.6357	1.1543	0.2826
LIVE Indicator	1	7.5925	96.1098	0.0062	0.9370

A method commonly used to develop useful models with quasi-complete separation of data points is eliminating the variable that is causing the problem (Allison 1999). Unfortunately, the live versus dead indicator is causing the quasi-complete separation of data points. As can be seen in table 4-69 all of the killed BG was identified. Removing that indicator parameter would defeat the purpose of the analysis. The root cause of the quasi-complete separation of data points is the fact that JBPDS identification performance for BG is virtually as good as its detection performance. This property is not universal for all agents or simulants. When testing whole systems, the distribution of concentration for identification opportunities, is constrained by detection performance. The identification process occurs only after detection. If detection does not occur, then there will not be an opportunity for identification. In the case of BG, the identification threshold is at or below the concentration for detection; hence most BG identification opportunities result in a positive identification.

Table 4-69 BG ASEC Identification Data Summery

Frequency Percent Row Pct Col Pct	Killed	Live	Total
Fail To Identify	0 0.00 0.00 0.00	6 10.34 100.00 28.57	6 10.34%
Identify	37 63.79 71.15 100.00	15 25.86 28.85 71.43	52 89.66%
Total	37 63.79%	21 36.21%	58 100.00%

The data set on BG identification in the ASEC is not suitable to support a comparison of JBPDS identification performance differences between live and killed BG.

Detection Performance in the ABT with Killed and Live Agent

Hypotheses 1 for detection in the ABT will be evaluated in this section. Hypotheses 1 compares the detection performance of JBPDS when challenged with live agent or live simulant with detection performance of JBPDS when challenged with that same agent or simulant after it has been killed. BG is the only live organism to be released in the ABT. Hence, this section will only compare live BG to Killed BG.

The following summary of results is developed in this section. The null hypothesis in hypothesis 1 is rejected for the live BG and Killed BG pair (P-value<0.0001). That is to say, for BG, JBPDS detection performance in the APT when challenged with live BG is statistically different from its performance when challenged with killed BG.

BG ABT detection results are based on 308 BG challenges at various concentrations of which 121 were detected and 187 were not detected.

The JBPDS BG detection model fit statistics are summarized in table 4-70. Clearly, at least one coefficient in the model is not equal to zero. However, a rescaled R-Square of 0.37 indicates that there is little predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 10.7663 with 7 degrees of freedom which produces a p-value of 0.2153. The deviance goodness of fit statistic is 305.87 with 288 degrees of freedom and a p-value of 0.2244. Neither goodness of fit tests are statistically significant which suggests that the model is not a bad fit.

Table 4-70 BG ABT Detection Model Fit Statistics for the full model

Criterion	Intercept Only	Intercept and Covariates
AIC	414.726	322.190
SC	418.456	337.110
-2 Log L	412.726	314.190
R-Square 0.2738	Max-rescaled R-Square 0.3709	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	98.5359	3	<.0001
Score	84.5047	3	<.0001
Wald	60.2490	3	<.0001

The intercept contributes to this model (p-values <0.001). The natural log of concentration contributes to this model (p-values <0.001). There is however no difference in the detection performance between the two JBPDSs (p-value=0.3343). As

can be seen in table 4-71, there is evidence that the detection performance of JBPDS when challenged with live BG is different from when it is challenged with dead BG. We conclude that in the ABT, JBPDS detection performance is different for killed and live BG (P-value<0.0001).

Table 4-71 BG ABT Detection Full Model Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	-2.0798	0.2581	64.9124	<.0001
Natural Log of Concentration	1	0.9349	0.1211	59.6222	<.0001
JBPDS Indicator	1	-0.1338	0.1386	0.9320	0.3343
LIVE or Killed BG Indicator	1	-0.7147	0.1659	18.5573	<.0001

Identification Performance in the ABT with Killed and Live Agent

Hypotheses 1 for identification in the ABT will be evaluated in this section. Hypotheses 1 compares the identification performance of JBPDS when challenged with live agent or live simulant with identification performance of JBPDS when challenged with that same agent or simulant after it has been killed. BG is the only live organism to be released in the ABT. Hence, this section will only compare live BG to Killed BG.

The following summary of results is developed in this section. Because of the narrow distribution of concentration, especially at the lower concentrations were identification tends to fail, no inferences are reached on hypothesis 1 for live and killed BG in the ABT.

BG ABT identification results are based on 121 BG challenges at various concentrations of which 117 were identified and 4 were not identified.

The BG model which is depicted in tables 4-72 and 4-73 includes an intercept, the natural log of the concentration, indicator parameters to identify the JBPDS, and an indicator parameter for living or dead BG. Regrettably, there was quasi-complete separation of data points in this model and hence, the maximum likelihood estimate may not exist. This model is not suited for analysis with logistic regression.

Table 4-72 ABT Identification Model Fit Statistics for the full model

Criterion	Intercept Only	Intercept and Covariates
AIC	37.142	30.471
SC	39.938	41.654
-2 Log L	35.142	22.471
R-Square 0.0994	Max-rescaled R-Square 0.3945	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	12.6715	3	0.0054
Score	7.8037	3	0.0502
Wald	5.7178	3	0.1262

Table 4-73 BG ABT Identification Full Model Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	6.7511	100.8	0.0045	0.9466
Natural Log of Concentration	1	1.3750	0.5847	5.5299	0.0187
JBPDS Indicator	1	0.7746	0.6737	1.3218	0.2503
LIVE or Killed BG Indicator	1	5.0561	100.8	0.0025	0.9600

A method commonly used to develop useful models with quasi-complete separation of data points is eliminating the variable that is causing the problem (Allison 1999). Unfortunately, the live versus dead indicator is causing the quasi-complete

separation of data points. As can be seen in table 4-74 all of the killed BG was identified. Removing that indicator parameter would defeat the purpose of the analysis. The root cause of the quasi-complete separation of data points is the fact that JBPDS identification performance for BG is virtually as good as its detection performance. This property is not universal for all agents or simulants. When testing whole systems, the distribution of concentration for identification opportunities, is constrained by detection performance. The identification process occurs only after detection. If detection does not occur, then there will not be an opportunity for identification. In the case of BG, the identification threshold is at or below the concentration for detection; hence most BG identification opportunities result in a positive identification.

Table 4-74 BG ABT Identification Data Summery

Frequency Percent Row Pct Col Pct	Killed	Live	Total
Fail To Identify	0 0.00 0.00 0.00	4 3.31 100.00 5.80	4 3.31%
Identify	52 42.98 44.44 100.00	65 53.72 55.56 94.20	117 96.69%
Total	52 42.98%	69 57.02%	121 100.00%

The data set on BG identification in the ABT is not suitable to support a comparison of JBPDS identification performance differences between live and killed BG.

Detection Performance in the Field with Killed and Live Agent

Hypotheses 1 for detection in the field will be evaluated in this section. Hypotheses 1 compares the detection performance of JBPDS when challenged with live agent or live simulant with detection performance of JBPDS when challenged with that same agent or simulant after it has been killed. BG is the only live organism to be released in the field. Hence, this section will only compare live BG to Killed BG.

The following summary of results is developed in this section. The null hypothesis in hypothesis 1 is rejected for the live BG and Killed BG pair (P-value=0.0030). That is to say, for BG, JBPDS detection performance in the field when challenged with live BG is statistically different from its performance when challenged with killed BG.

BG Field detection results are based on 188 BG challenges at various concentrations of which 153 were detected and 35 were not detected.

The BG model which is depicted in tables 4-75 and 4-76 includes an intercept, the natural log of the concentration, indicator parameters to identify the JBPDS, and an indicator parameter for living or dead BG. Regrettably, there was quasi-complete separation of data points in this model and hence, the maximum likelihood estimate may not exist. This model is not suited for analysis with logistic regression.

Table 4-75 BG Field Detection Model Fit Statistics for the full model

Criterion	Intercept Only	Intercept and Covariates
AIC	182.714	182.191
SC	185.950	211.319
-2 Log L	180.714	164.191
R-Square 0.0841	Max-rescaled R-Square 0.1362	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	16.5231	8	0.0355
Score	13.1132	8	0.1080
Wald	9.5227	8	0.3001

Table 4-76 BG Field Detection Full Model Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	-22.9931	509.5	0.0020	0.9640
Natural Log of Concentration	1	0.1111	0.1008	1.2152	0.2703
JBPDS_1	1	5.9836	127.4	0.0022	0.9625
JBPDS_3	1	6.2237	127.4	0.0024	0.9610
JBPDS_4	1	6.1464	127.4	0.0023	0.9615
JBPDS_5	1	6.0866	127.4	0.0023	0.9619
JBPDS_6	1	5.9073	127.4	0.0022	0.9630
JBPDS_7	1	6.1172	127.4	0.0023	0.9617
LIVE Indicator	1	0.6823	0.2337	8.5213	0.0035

A method commonly used to develop useful models with quasi-complete separation of data points is eliminating the variable that is causing the problem (Allison 1999). The indicator parameters that identify the JBPDS are the cause of the quasi-complete separation of data points. These indicator parameters were included in the model to control for variation between different JBPDS. As a result of correcting for quasi-complete separation of data points, the analysis of the effect of living versus dead BG on detection performance is based on a simpler model which is limited to an

intercept, the natural log of the concentration, and an indicator parameter for living or dead BG. The simpler model is described below.

The JBPDS BG detection model fit statistics for the reduced field model are summarized in table 4-77. Clearly, at least one coefficient in the model is not equal to zero. However, a rescaled R-Square of 0.09 indicates that there is little predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 13.54 with 7 degrees of freedom which produces a p-value of 0.06. The deviance goodness of fit statistic is 94.14 with 83 degrees of freedom and a p-value of 0.19. Both goodness of fit tests are statistically significant or nearly statistically significant which suggests that the model is a poor fit.

Table 4-77 BG Field Detection Model Fit Statistics for the reduced model

Criterion	Intercept Only	Intercept and Covariates
AIC	182.714	176.125
SC	185.950	185.835
-2 Log L	180.714	170.125
R-Square 0.0548	Max-rescaled R-Square 0.0887	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	10.5886	2	0.0050
Score	9.9356	2	0.0070
Wald	9.0970	2	0.0106

The intercept contributes to this model (p-values <0.0001). The natural log of concentration does not have a statistically significant contribution to this model (p-value =0.3316). Never the less it is intuitive that detection performance will increase with increased concentration. As can be seen in table 4-78, there is evidence that the detection

performance of JBPDS when challenged with live BG is different from when it is challenged with dead BG. We conclude that in the field, JBPDS detection performance is different for killed and live BG (P-value=0.0030).

Table 4-78 BG Field Detection Full Model Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	1.4415	0.3397	18.0067	<.0001
Natural Log of Concentration	1	0.0949	0.0977	0.9427	0.3316
LIVE Indicator	1	0.6793	0.2290	8.7979	0.0030

Identification Performance in the Field with Killed and Live Simulant

Hypotheses 1 for identification in the field will be evaluated in this section. Hypotheses 1 compares the identification performance of JBPDS when challenged with live agent or live simulant with detection performance of JBPDS when challenged with that same agent or simulant after it has been killed. BG is the only live organism to be released in the field. Hence, this section will only compare live BG to Killed BG.

The following summary of results is developed in this section. The null hypothesis in hypothesis 1 is rejected for the live BG and Killed BG pair (P-value<0.0001).

That is to say, for BG, JBPDS identification performance in the field when challenged with live BG is statistically different from its performance when challenged with the killed BG.

BG field identification results are based on 153 BG challenges at various concentrations of which 99 were detected and 54 were not detected.

The JBPDS BG identification model fit statistics for the field are summarized in table 4-79. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-Square of 0.26 indicates that there is marginal predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 4.44 with 8 degrees of freedom which produces a p-value of 0.81. The deviance goodness of fit statistic is 138.37 with 125 degrees of freedom and a p-value of 0.19. Neither goodness of fit tests are statistically significant which suggests that the model is not a bad fit.

Table 4-79 BG Field Detection Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	200.670	184.101
SC	203.700	211.375
-2 Log L	198.670	166.101
R-Square 0.1917	Max-rescaled R-Square 0.2637	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	32.5685	8	<.0001
Score	29.9152	8	0.0002
Wald	24.4198	8	0.0019

The intercept does not statistically contribute to this model (p-values =0.6202). The natural log of concentration does have a statistically significant contribution to this model (p-value =0.0015). As can be seen in table 4-80, there is evidence that the identification performance of JBPDS when challenged with live BG is different from when it is challenged with dead BG. We conclude that in the field, JBPDS identification performance is different for killed and live BG (P-value<0.0001).

Table 4-80 BG Field Identification Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	0.7430	1.4995	0.2455	0.6202
Natural Log of Concentration	1	0.3457	0.1087	10.1238	0.0015
JBPDS_1	1	-0.0630	0.4169	0.0229	0.8798
JBPDS_3	1	-0.7847	0.4766	2.7103	0.0997
JBPDS_4	1	0.2537	0.4094	0.3840	0.5355
JBPDS_5	1	-0.0980	0.4111	0.0568	0.8117
JBPDS_6	1	-0.5242	0.4115	1.6226	0.2027
JBPDS_7	1	-0.1416	0.3999	0.1253	0.7233
LIVE Indicator	1	0.8188	0.2067	15.6926	<.0001

Chapter 5 Chamber Comparison

This chapter will focus on the effect different chambers have on detector performance. Of special interest is comparing the detector performance in the CAC to detector performance in the field. If performance in the CAC were identical to performance in the field, there would be no need to conduct field tests and we would not need to be concerned about agent simulant relationships. Specifically, this chapter provides results and discussions for the following hypothesis for both the detect function and the identification function.

Hypothesis 4:

- H_o: JBPDS performance is the same in all chambers (CAC, ASEC, ABT, and the field)
- H_a: JBPDS performance is not the same in all chambers (CAC, ASEC, ABT, and the field)

The following detection observations are developed in this chapter:

- For killed BG detection performance in the field differs from the other chambers (p-value<0.0001)
- For live BG detection performance in the field and ABT are different from the other chambers (p-value=0.0143 and 0.0039 respectively)
- For Le ALO detection performance in the field and ABT are different from the other chambers (p-value=0.0100 and 0.0003 respectively)

- For N ALO detection performance in the field, ABT, and CAC differ from the other chambers (p-value<0.0001, 0.0102, and <0.0112 respectively)
- For Nu ALO detection performance in the field and ABT are different from the other chambers (p-value=0.0100 and 0.0003 respectively)
- For denatured Xr detection performance in the field, ABT, and CAC differ from the other chambers (p-value<0.0001, <0.0001, and <0.0001 respectively)

The following identification observations are developed in this chapter:

- For Nu ALO identification performance in the field, ABT, and CAC are different from the other chambers (p-value=0.0034, 0.0075, and 0.0004 respectively)

Clearly, detection and identification performance are different in different chambers. One can not simply assume CAC detection and identification results are representative of field performance.

The effect of the different chambers on detection performance is an order of magnitude greater than the effect of the live indicator.

Detection Performance Across Chambers

The purpose of this section is to show the effect different chambers have on the detection performance of JBPDS. Of special interest is comparing detection performance in the CAC detection performance in the field.

The conclusion of this section is that detection performance is influenced by the chamber in which the test is conducted. Specific detection observations made in this section are:

- For killed BG detection performance in the field differs from the other chambers (p-value<0.0001)
- For live BG detection performance in the field and ABT are different from the other chambers (p-value=0.0143 and 0.0039 respectively)
- For Le ALO detection performance in the field and ABT are different from the other chambers (p-value=0.0100 and 0.0003 respectively)
- For N ALO detection performance in the field, ABT, and CAC differ from the other chambers (p-value<0.0001, 0.0102, and <0.0112 respectively)
- For Nu ALO detection performance in the field and ABT are different from the other chambers (p-value=0.0100 and 0.0003 respectively)
- For denatured Xr detection performance in the field, ABT, and CAC differ from the other chambers (p-value<0.0001, <0.0001, and <0.0001 respectively)

Killed BG detection results for the field, ABT, ASEC, and CAC are based on 291 BG challenges at various concentrations of which 178 were detected and 113 were not detected.

The full killed BG model which is depicted in tables 5-1 and 5-2 includes an intercept, the natural log of the concentration, indicator parameters for the JBPDSs, and indicator parameters for the chambers. Regrettably, there was quasi-complete separation of data points in this model and hence, the maximum likelihood estimate may not exist. This model is not suited for analysis with logistic regression.

Table 5-1 Killed BG CAC-ASEC-ABT-Field Full Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	390.770	266.566
SC	394.443	314.320
-2 Log L	388.770	240.566
R-Square 0.3991	Max-rescaled R-Square 0.5414	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF Pr > ChiSq
Likelihood Ratio	148.2031	12 <.0001
Score	116.0629	12 <.0001
Wald	64.8963	12 <.0001

Table 5-2 Killed BG CAC-ASEC-ABT-Field Full Model Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	13.2643	514.5	0.0007	0.9794
Natural Log of Concentration	1	0.8891	0.1301	46.6880	<.0001
FIELD Indicator	1	-2.2830	0.6238	13.3963	0.0003
ABT Indicator	1	-0.2461	0.2892	0.7241	0.3948
CAC Indicator	1	0.1573	0.3193	0.2426	0.6224
JBPDS2 Indicator	1	0.0977	0.3127	0.0975	0.7548
JBPDS3 Indicator	0	0	.	.	.
JBPDS4 Indicator	1	0.4180	0.2332	3.2139	0.0730
JBPDS5 Indicator	0	0	.	.	.
JBPDS6 Indicator	1	-5.4183	287.3	0.0004	0.9850
JBPDS7 Indicator	1	-5.3313	337.3	0.0002	0.9874
JBPDS8 Indicator	1	1.5534	0.6631	5.4876	0.0192
JBPDS9 Indicator	1	1.2750	0.6901	3.4135	0.0647
JBPDS10 Indicator	1	0.00447	0.7653	0.0000	0.9953
JBPDS11 Indicator	1	-6.2647	261.4	0.0006	0.9809
JBPDS12 Indicator	0	0	.	.	.

A method commonly used to develop useful models with quasi-complete separation of data points is eliminating the variable that is causing the problem (Allison 1999). The indicator parameters that identify the JBPDS are the cause of the quasi-

complete separation of data points. These indicator parameters were included in the model to control for variation between different JBPDS. As a result of correcting for quasi-complete separation of data points, the analysis of the chamber effect on detection performance is based on a simpler model which is limited to an intercept, the natural log of the concentration, and indicator parameters for the different chambers. The simpler model is described below.

The JBPDS Killed BG detection model fit statistics for the reduced CAC, ASEC, ABT, and field model are summarized in table 5-3. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-Square of 0.49 indicates that there is minimal predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 155.6895 with 8 degrees of freedom which produces a p-value less than 0.0001. The deviance goodness of fit statistic is 212.8583 with 143 degrees of freedom and a p-value of 0.0001. Both goodness of fit tests are statistically significant statistically significant which suggests that the model is a poor fit.

Table 5-3 Killed BG CAC-ASEC-ABT-Field Reduced Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	390.770	269.680
SC	394.443	288.046
-2 Log L	388.770	259.680

R-Square 0.3583 Max-rescaled R-Square 0.4861

Testing Global Null Hypothesis: All coefficients=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	129.0899	4	<.0001
Score	105.8286	4	<.0001
Wald	71.1084	4	<.0001

The intercept and the natural log of concentration both contribute to this model (p-values of 0.0702 and <0.0001 respectively). As can be seen in table 5-4, killed BG detection performance in the field is statistically different from killed BG detection performance in the other chambers (p-values<0.0001). The difference in the shift parameters between field and CAC is 1.8410.

Table 5-4 Killed BG CAC-ASEC-ABT-Field Reduced Model Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	-0.7525	0.4157	3.2775	0.0702
Natural Log of Concentration	1	0.7688	0.1137	45.7085	<.0001
FIELD Indicator	1	-1.7446	0.3061	32.4859	<.0001
ABT Indicator	1	-0.0175	0.2022	0.0075	0.9312
CAC Indicator	1	0.0964	0.2714	0.1261	0.7225

Live BG detection results for the field, ABT, ASEC, and CAC are based on 346 BG challenges at various concentrations of which 179 were detected and 167 were not detected.

The JBPDS live BG detection model fit statistics for the field, ABT, ASEC, and CAC are summarized in table 5-5. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-Square of 0.30 indicates that there is minimal predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 27.1860 with 8 degrees of freedom which produces a p-value of 0,0007. The deviance goodness of fit statistic is 366.0084 with 300 degrees of freedom and a p-value of 0.0054. Both goodness of fit tests are statistically significant which suggests that the model is a not good fit.

Table 5-5 Live BG CAC-ASEC-ABT-Field Full Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	481.242	418.008
SC	485.088	468.012
-2 Log L	479.242	392.008
R-Square 0.2228	Max-rescaled R-Square 0.2973	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF Pr > ChiSq
Likelihood Ratio	87.2334	12 <.0001
Score	72.6926	12 <.0001
Wald	51.9164	12 <.0001

The intercept does not contribute statistically to this model (p-value=0.9839). The natural log of concentration contributes to this model (p-values <.0001). As can be seen in table 5-6, live BG detection performance in the field is statistically different from killed BG detection performance in the other chambers (p-value=0.0143). The difference in the shift parameters between field and CAC is 0.5900.

Table 5-6 Live BG CAC-ASEC-ABT-Field Full Model Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	8.6319	428.0	0.0004	0.9839
Natural Log of Concentration	1	0.6252	0.1091	32.8480	<.0001
FIELD Indicator	1	-0.8707	0.3553	6.0034	0.0143
ABT Indicator	1	-0.8283	0.2870	8.3314	0.0039
CAC Indicator	1	-0.2807	0.3337	0.7078	0.4002
JBPDS2 Indicator	1	-0.7010	0.3291	4.5387	0.0331
JBPDS3 Indicator	0	0	.	.	.
JBPDS4 Indicator	1	- 0.0267	0.1635	0.0267	0.8701
JBPDS5 Indicator	0	0	.	.	.
JBPDS6 Indicator	1	-0.1618	0.4121	0.1541	0.6946
JBPDS7 Indicator	1	-7.6220	428.0	0.0003	0.9858
JBPDS8 Indicator	1	-0.3088	0.4632	0.4444	0.5050
JBPDS9 Indicator	1	-0.2286	0.4217	0.2940	0.5876
JBPDS10 Indicator	1	-0.1965	0.3968	0.2452	0.6205
JBPDS11 Indicator	1	-0.1270	0.3869	0.1078	0.7427
JBPDS12 Indicator	0	0	.	.	.

Killed LE ALO detection results for the field, ABT, ASEC, and CAC are based on 195 LE ALO challenges at various concentrations.

The full killed LE ALO model which is depicted in tables 5-7 and 5-8 includes an intercept, the natural log of the concentration, indicator parameters for the JBPDSs, and indicator parameters for the chambers. Regrettably, there was quasi-complete separation

of data points in this model and hence, the maximum likelihood estimate may not exist.

This model is not suited for analysis with logistic regression.

Table 5-7 Killed LE ALO CAC-ASEC-ABT-Field Full Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	188.545	131.270
SC	191.818	173.819
-2 Log L	186.545	105.270
R-Square 0.3408	Max-rescaled R-Square 0.5535	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	81.2753	12	<.0001
Score	53.5532	12	<.0001
Wald	24.5614	12	0.0170

Table 5-8 Killed LE ALO CAC-ASEC-ABT-Field Full Model Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	590.9	0.0018	0.9665
Natural Log of Concentration	1	0.5093	22.6233	<.0001
FIELD Indicator	1	1.0416	14.1662	0.0002
ABT Indicator	1	499.6	0.0005	0.9829
CAC Indicator	1	0.3751	1.9338	0.1643
JBPDS2 Indicator	1	0.3345	2.5267	0.1119
JBPDS3 Indicator	0	0	.	.
JBPDS4 Indicator	1	706.6	0.0000	1.0000
JBPDS5 Indicator	0	0	.	.
JBPDS6 Indicator	1	198.9	0.0014	0.9702
JBPDS7 Indicator	1	244.8	0.0009	0.9757
JBPDS8 Indicator	1	0.8044	1.3806	0.2400
JBPDS9 Indicator	1	0.7196	0.3941	0.5301
JBPDS10 Indicator	1	0.6975	2.2298	0.1354
JBPDS11 Indicator	1	0.7850	0.2679	0.6047
JBPDS12 Indicator	0	0	.	.

A method commonly used to develop useful models with quasi-complete separation of data points is eliminating the variable that is causing the problem (Allison 1999). Regrettably, simpler models also had quasi-complete separation of data points.

Killed N ALO detection results for the field, ABT, ASEC, and CAC are based on 491 N ALO challenges at various concentrations.

The full killed N ALO model which is depicted in tables 5-9 and 5-10 includes an intercept, the natural log of the concentration, indicator parameters for the JBPDSs, and indicator parameters for the chambers. Regrettably, there was quasi-complete separation of data points in this model and hence, the maximum likelihood estimate may not exist. This model is not suited for analysis with logistic regression.

Table 5-9 Killed N ALO CAC-ASEC-ABT-Field Full Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	666.449	576.385
SC	670.645	630.939
-2 Log L	664.449	550.385
R-Square 0.2073	Max-rescaled R-Square 0.2795	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	114.0637	12	<.0001
Score	88.8449	12	<.0001
Wald	60.9744	12	<.0001

Table 5-10 Killed N ALO CAC-ASEC-ABT-Field Full Model Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	280.2	0.0013	0.9709
Natural Log of Concentration	1	0.0688	38.0849	<.0001
FIELD Indicator	1	0.3991	12.0648	0.0005
ABT Indicator	1	0.2318	5.6572	0.0174
CAC Indicator	1	0.2783	8.6846	0.0032
JBPDS2 Indicator	1	0.2856	2.7176	0.0992
JBPDS3 Indicator	0	.	.	.
JBPDS4 Indicator	1	0.1246	2.2087	0.1372
JBPDS5 Indicator	0	.	.	.
JBPDS6 Indicator	1	0.5209	0.5658	0.4519
JBPDS7 Indicator	1	0.6514	0.7820	0.3765
JBPDS8 Indicator	1	280.2	0.0007	0.9789
JBPDS9 Indicator	1	0.4746	0.3588	0.5492
JBPDS10 Indicator	1	0.5097	1.3983	0.2370
JBPDS11 Indicator	1	0.4878	0.0568	0.8116
JBPDS12 Indicator	0	.	.	.

A method commonly used to develop useful models with quasi-complete separation of data points is eliminating the variable that is causing the problem (Allison 1999). The indicator parameters that identify the JBPDS are the cause of the quasi-complete separation of data points. These indicator parameters were included in the model to control for variation between different JBPDS. As a result of correcting for quasi-complete separation of data points, the analysis of the chamber effect on detection performance is based on a simpler model which is limited to an intercept, the natural log of the concentration, and indicator parameters for the different chambers. The simpler model is described below.

The JBPDS Killed N ALO detection model fit statistics for the reduced CAC, ASEC, ABT, and field model are summarized in table 5-11. Clearly, at least one

coefficient in the model is not equal to zero. A rescaled R-Square of 0.25 indicates that there is minimal predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 124.4851 with 8 degrees of freedom which produces a p-value less than 0.0001. The deviance goodness of fit statistic is 453.9286 with 229 degrees of freedom and a p-value less than 0.0001. Both goodness of fit tests are statistically significant statistically significant which suggests that the model is a poor fit.

Table 5-11 Killed N ALO CAC-ASEC-ABT-Field Reduced Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	666.449	574.232
SC	670.645	595.214
-2 Log L	664.449	564.232
R-Square 0.1846	Max-rescaled R-Square 0.2490	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	100.2166	4	<.0001
Score	78.6144	4	<.0001
Wald	59.9043	4	<.0001

The intercept does not contribute statistically to this model (p-value=0.1155). The natural log of concentration contributes to this model (p-values <.0001). As can be seen in table 5-12, killed N ALO detection performance in the field is statistically different from killed N ALO detection performance in the other chambers (p-value<0.001). The difference in the shift parameters between field and CAC is 0.9674.

Table 5-12 Killed N ALO CAC-ASEC-ABT-Field Reduced Model Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	0.3111	2.4768	0.1155
Natural Log of Concentration	1	0.0685	35.2552	<.0001
FIELD Indicator	1	0.2266	46.1487	<.0001
ABT Indicator	1	0.1521	6.5917	0.0102
CAC Indicator	1	0.2254	6.4386	0.0112

Killed NU ALO detection results for the field, ABT, ASEC, and CAC are based on 375 killed NU ALO challenges at various concentrations.

The full killed NU ALO model which is depicted in tables 5-13 and 5-14 includes an intercept, the natural log of the concentration, indicator parameters for the JBPDSs, and indicator parameters for the chambers. Regrettably, there was quasi-complete separation of data points in this model and hence, the maximum likelihood estimate may not exist. This model is not suited for analysis with logistic regression.

Table 5-13 Killed NU ALO CAC-ASEC-ABT-Field Full Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	497.646	391.076
SC	501.567	442.056
-2 Log L	495.646	365.076

R-Square 0.2953 Max-rescaled R-Square 0.4017

Testing Global Null Hypothesis: All coefficients=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	130.5701	12	<.0001
Score	104.1044	12	<.0001
Wald	59.7900	12	<.0001

Table 5-14 Killed NU ALO CAC-ASEC-ABT-Field Full Model Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	1268.2	0.0000	0.9945
Natural Log of Concentration	1	0.0793	51.4387	<.0001
FIELD Indicator	1	0.4849	1.1730	0.2788
ABT Indicator	1	0.2252	7.9299	0.0049
CAC Indicator	1	0.2632	0.0124	0.9115
JBPDS2 Indicator	1	0.1848	1.2117	0.2710
JBPDS3 Indicator	0	.	.	.
JBPDS4 Indicator	1	0.2065	1.0510	0.3053
JBPDS5 Indicator	0	.	.	.
JBPDS6 Indicator	1	0.7241	1.9963	0.1577
JBPDS7 Indicator	1	578.3	0.0002	0.9891
JBPDS8 Indicator	1	0.7069	0.1895	0.6633
JBPDS9 Indicator	1	431.9	0.0003	0.9868
JBPDS10 Indicator	1	1042.8	0.0000	0.9945
JBPDS11 Indicator	1	0.6120	0.3595	0.5488
JBPDS12 Indicator	0	.	.	.

A method commonly used to develop useful models with quasi-complete separation of data points is eliminating the variable that is causing the problem (Allison 1999). The indicator parameters that identify the JBPDS are the cause of the quasi-complete separation of data points. These indicator parameters were included in the model to control for variation between different JBPDS. As a result of correcting for quasi-complete separation of data points, the analysis of the chamber effect on detection performance is based on a simpler model which is limited to an intercept, the natural log of the concentration, and indicator parameters for the different chambers. The simpler model is described below.

The JBPDS Killed NU ALO detection model fit statistics for the reduced CAC, ASEC, ABT, and field model are summarized in table 5-15. Clearly, at least one

coefficient in the model is not equal to zero. A rescaled R-Square of 0.36 indicates that there is minimal predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 24.0662 with 8 degrees of freedom which produces a p-value of 0.0022. The deviance goodness of fit statistic is 269.3912 with 156 degrees of freedom and a p-value of less than 0.0001. Both goodness of fit tests are statistically significant statistically significant which suggests that the model is a poor fit.

Table 5-15 Killed NU ALO CAC-ASEC-ABT-Field Reduced Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	497.646	391.445
SC	501.567	411.053
-2 Log L	495.646	381.445
R-Square 0.2637	Max-rescaled R-Square 0.3587	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	114.2003	4	<.0001
Score	90.6992	4	<.0001
Wald	58.0144	4	<.0001

The intercept does not contribute statistically to this model (p-value=0.2344). The natural log of concentration contributes to this model (p-values <.0001). As can be seen in table 5-16, killed NU detection performance in the field is statistically different from killed NU detection performance in the other chambers (p-value=0.0100). The difference in the shift parameters between field and CAC is 0.7750.

Table 5-16 Killed NU ALO CAC-ASEC-ABT-Field Reduced Model Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	0.3257	1.4139	0.2344
Natural Log of Concentration	1	0.0795	53.2413	<.0001
FIELD Indicator	1	0.2514	6.6290	0.0100
ABT Indicator	1	0.1812	13.1191	0.0003
CAC Indicator	1	0.2482	0.2654	0.6065

Denatured XR detection results for the field, ABT, ASEC, and CAC are based on 207 denatured XR challenges at various concentrations.

The full denatured XR model which is depicted in tables 5-17 and 5-18 includes an intercept, the natural log of the concentration, indicator parameters for the JBPDSs, and indicator parameters for the chambers. Regrettably, there was quasi-complete separation of data points in this model and hence, the maximum likelihood estimate may not exist. This model is not suited for analysis with logistic regression.

Table 5-17 Denatured XR CAC-ASEC-ABT-Field Full Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	283.017	96.298
SC	286.349	132.958
-2 Log L	281.017	74.298

R-Square 0.6316 Max-rescaled R-Square 0.8504

Testing Global Null Hypothesis: All coefficients=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	206.7187	10	<.0001
Score	119.3007	10	<.0001
Wald	35.0014	10	0.0001

Table 5-18 Denatured XR CAC-ASEC-ABT-Field Full Model Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	499.8	0.0006	0.9812
Natural Log of Concentration	1	0.7828	33.9661	<.0001
FIELD Indicator	1	151.2	0.0132	0.9085
ABT Indicator	1	0.6515	13.7072	0.0002
CAC Indicator	1	0.6803	10.1991	0.0014
JBPDS2 Indicator	1	0.5477	10.0128	0.0016
JBPDS3 Indicator	0	.	.	.
JBPDS4 Indicator	1	0.4617	2.8395	0.0920
JBPDS5 Indicator	0	.	.	.
JBPDS7 Indicator	1	193.7	0.0000	0.9992
JBPDS8 Indicator	1	193.7	0.0000	0.9992
JBPDS9 Indicator	1	151.2	0.0017	0.9667
JBPDS11 Indicator	1	193.7	0.0000	0.9992
JBPDS12 Indicator	0	.	.	.

A method commonly used to develop useful models with quasi-complete separation of data points is eliminating the variable that is causing the problem (Allison 1999). The indicator parameters that identify the JBPDS are the cause of the quasi-complete separation of data points. These indicator parameters were included in the model to control for variation between different JBPDS. As a result of correcting for quasi-complete separation of data points, the analysis of the chamber effect on detection performance is based on a simpler model which is limited to an intercept, the natural log of the concentration, and indicator parameters for the different chambers. The simpler model is described below.

The JBPDS denatured XR detection model fit statistics for the reduced CAC, ASEC, ABT, and field model are summarized in table 5-19. Clearly, at least one

coefficient in the model is not equal to zero. A rescaled R-Square of 0.8017 indicates that there is predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 11.6897 with 7 degrees of freedom which produces a p-value of 0.1112. The deviance goodness of fit statistic is 55.4268 with 106 degrees of freedom and a p-value of unity. Neither goodness of fit test is statistically significant which suggests that the model is not a bad fit.

Table 5-19 Denatured XR CAC-ASEC-ABT-Field Reduced Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	283.017	103.702
SC	286.349	120.365
-2 Log L	281.017	93.702
R-Square 0.5954	Max-rescaled R-Square 0.8017	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	187.3146	4	<.0001
Score	113.7123	4	<.0001
Wald	37.7063	4	<.0001

The intercept and the natural log of concentration both contributes to this model (p-values of <.0001 and <.0001 respectively). As can be seen in table 5-20, denatured XR performance in the field is statistically different from denatured XR detection performance in the other chambers (p-value<0.0001). The difference in the shift parameters between field and CAC is 7.3452.

Table 5-20 Denatured XR CAC-ASEC-ABT-Field Reduced Model Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	2.1253	24.3856	<.0001
Natural Log of Concentration	1	0.6445	35.1309	<.0001
FIELD Indicator	1	1.6111	37.2393	<.0001
ABT Indicator	1	0.5161	21.3665	<.0001
CAC Indicator	1	0.6153	16.3276	<.0001

Identification Performance Across Chambers

The purpose of this section is to show the effect different chambers have on the detection performance of JBPDS. Of special interest is comparing detection performance in the CAC detection performance in the field.

The conclusion of this section is that identification performance is influenced by the chamber in which the test is conducted. Specific identification observations made in this section are: For Nu ALO identification performance in the field, ABT, and CAC are different from the other chambers (p-value=0.0034, 0.0075, and 0.0004 respectively)

Killed BG results for the field, ABT, ASEC, and CAC are based on 178 killed BG challenges at various concentrations of which 162 were identified and 16 were not identified.

The full killed BG identification model which is depicted in tables 5-21 and 5-22 includes an intercept, the natural log of the concentration, indicator parameters for the JBPDSs, and indicator parameters for the chambers. Regrettably, there was quasi-complete separation of data points in this model and hence, the maximum likelihood estimate may not exist. This model is not suited for analysis with logistic regression.

Table 5-21 Killed BG CAC-ASEC-ABT-Field Full Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	109.611	91.482
SC	112.793	132.845
-2 Log L	107.611	65.482
R-Square 0.2108	Max-rescaled R-Square 0.4645	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	42.1288	12	<.0001
Score	45.4914	12	<.0001
Wald	6.0529	12	0.9134

Table 5-22 Killed BG CAC-ASEC-ABT-Field Full Model Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	9.2282	186.1	0.0025	0.9605
Natural Log of Concentration	1	0.1893	0.2465	0.5901	0.4424
FIELD Indicator	1	5.3099	71.4037	0.0055	0.9407
ABT Indicator	1	-0.1081	91.1663	0.0000	0.9991
CAC Indicator	1	0.2362	94.8427	0.0000	0.9980
JBPDS2 Indicator	1	0.2080	93.2334	0.0000	0.9982
JBPDS3 Indicator	0	0	.	.	.
JBPDS4 Indicator	1	0.0725	85.4766	0.0000	0.9993
JBPDS5 Indicator	0	0	.	.	.
JBPDS6 Indicator	1	0.5250	0.5897	0.7929	0.3732
JBPDS7 Indicator	1	-4.6670	51.6480	0.0082	0.9280
JBPDS8 Indicator	1	0.2398	0.5826	0.1695	0.6806
JBPDS9 Indicator	1	0.9503	0.5428	3.0657	0.0800
JBPDS10 Indicator	1	0.4058	0.5314	0.5832	0.4451
JBPDS11 Indicator	1	-0.4227	0.6511	0.4214	0.5163
JBPDS12 Indicator	0	0	.	.	.

A method commonly used to develop useful models with quasi-complete separation of data points is eliminating the variable that is causing the problem (Allison

1999). Unfortunately, removing variables failed to eliminate quasi-complete separation of data points. Neither the full nor the reduced model is suitable for analysis.

Live BG results for the field, ABT, ASEC, and CAC are based on 179 live BG challenges at various concentrations of which 131 were identified and 48 were not identified.

The full live BG identification model which is depicted in tables 5-23 and 5-24 includes an intercept, the natural log of the concentration, indicator parameters for the JBPDSs, and indicator parameters for the chambers. Regrettably, there was quasi-complete separation of data points in this model and hence, the maximum likelihood estimate may not exist. This model is not suited for analysis with logistic regression.

Table 5-23 Live BG CAC-ASEC-ABT-Field Full Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	210.147	159.307
SC	213.335	200.743
-2 Log L	208.147	133.307
R-Square 0.3417	Max-rescaled R-Square 0.4971	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	74.8406	12	<.0001
Score	66.7405	12	<.0001
Wald	34.2775	12	0.0006

Table 5-24 Live BG CAC-ASEC-ABT-Field Full Model Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	1.9511	421.6	0.0000	0.9963
Natural Log of Concentration	1	0.4758	0.1458	10.6486	0.0011
FIELD Indicator	1	1.0854	0.6208	3.0571	0.0804
ABT Indicator	1	-0.8007	0.6444	1.5439	0.2140
CAC Indicator	1	-6.2916	235.7	0.0007	0.9787
JBPDS2 Indicator	1	0.5682	0.6084	0.8724	0.3503
JBPDS3 Indicator	0	0	.	.	.
JBPDS4 Indicator	1	-0.5865	0.5944	0.9735	0.3238
JBPDS5 Indicator	0	0	.	.	.
JBPDS6 Indicator	1	-0.3307	0.4502	0.5394	0.4627
JBPDS7 Indicator	1	6.8923	349.5	0.0004	0.9843
JBPDS8 Indicator	1	-1.5192	0.6413	5.6112	0.0178
JBPDS9 Indicator	1	-0.1315	0.4482	0.0861	0.7692
JBPDS10 Indicator	1	-0.2174	0.4397	0.2444	0.6210
JBPDS11 Indicator	1	-0.5132	0.4240	1.4651	0.2261
JBPDS12 Indicator	0	0	.	.	.

A method commonly used to develop useful models with quasi-complete separation of data points is eliminating the variable that is causing the problem (Allison 1999). Unfortunately, removing variables failed to eliminate quasi-complete separation of data points. Neither the full nor the reduced model is suitable for analysis.

Killed LE ALO results for the field, ABT, ASEC, and CAC are based on 157 killed LE ALO challenges at various concentrations.

The full killed LE ALO identification model which is depicted in tables 5-25 and 5-26 includes an intercept, the natural log of the concentration, indicator parameters for the JBPDSs, and indicator parameters for the chambers. Regrettably, there was quasi-complete separation of data points in this model and hence, the maximum likelihood estimate may not exist. This model is not suited for analysis with logistic regression.

Table 5-25 Killed LE ALO CAC-ASEC-ABT-Field Full Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	205.360	177.347
SC	208.416	217.079
-2 Log L	203.360	151.347
R-Square 0.2820	Max-rescaled R-Square 0.3883	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	52.0126	12	<.0001
Score	44.3664	12	<.0001
Wald	26.8547	12	0.0081

Table 5-26 Killed LE ALO CAC-ASEC-ABT-Field Full Model Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	489.5	0.0029	0.9574
Natural Log of Concentration	1	0.1582	23.4315	<.0001
FIELD Indicator	1	0.5770	4.7941	0.0286
ABT Indicator	1	392.5	0.0001	0.9906
CAC Indicator	1	0.3199	2.9860	0.0840
JBPDS2 Indicator	1	0.2383	0.0150	0.9024
JBPDS3 Indicator	0	.	.	.
JBPDS4 Indicator	1	555.1	0.0000	1.0000
JBPDS5 Indicator	0	.	.	.
JBPDS6 Indicator	1	0.7305	0.0343	0.8531
JBPDS7 Indicator	1	195.1	0.0009	0.9767
JBPDS8 Indicator	1	155.2	0.0015	0.9687
JBPDS9 Indicator	1	153.0	0.0016	0.9681
JBPDS10 Indicator	1	0.7530	0.0290	0.8648
JBPDS11 Indicator	1	0.7285	0.4727	0.4918
JBPDS12 Indicator	0	.	.	.

A method commonly used to develop useful models with quasi-complete separation of data points is eliminating the variable that is causing the problem (Allison 1999). Unfortunately, removing variables failed to eliminate quasi-complete separation

of data points. Neither the full nor the reduced model is suitable for analysis with logistic regression.

Killed N ALO results for the field, ABT, ASEC, and CAC are based on 288 killed N ALO challenges at various concentrations.

The full killed N ALO identification model which is depicted in tables 5-27 and 5-28 includes an intercept, the natural log of the concentration, indicator parameters for the JBPDSs, and indicator parameters for the chambers. Regrettably, there was quasi-complete separation of data points in this model and hence, the maximum likelihood estimate may not exist. This model is not suited for analysis with logistic regression.

Table 5-27 Killed N ALO CAC-ASEC-ABT-Field Full Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	270.959	236.386
SC	274.621	284.005
-2 Log L	268.959	210.386
R-Square 0.1840	Max-rescaled R-Square 0.3032	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	58.5724	12	<.0001
Score	61.0014	12	<.0001
Wald	36.9753	12	0.0002

Table 5-28 Killed N ALO CAC-ASEC-ABT-Field Full Model Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	240.4	0.0001	0.9927
Natural Log of Concentration	1	0.0689	20.4083	<.0001
FIELD Indicator	1	105.9	0.0033	0.9543
ABT Indicator	1	105.9	0.0029	0.9571
CAC Indicator	1	106.0	0.0023	0.9620
JBPDS2 Indicator	1	132.2	0.0000	0.9992
JBPDS3 Indicator	0	.	.	.
JBPDS4 Indicator	1	0.2330	0.0937	0.7595
JBPDS5 Indicator	0	.	.	.
JBPDS6 Indicator	1	0.4564	0.9826	0.3216
JBPDS7 Indicator	1	0.6745	0.1922	0.6611
JBPDS8 Indicator	1	0.5492	0.0466	0.8291
JBPDS9 Indicator	1	81.4004	0.0047	0.9451
JBPDS10 Indicator	1	0.4498	0.1914	0.6618
JBPDS11 Indicator	1	0.4603	0.9100	0.3401
JBPDS12 Indicator	0	.	.	.

A method commonly used to develop useful models with quasi-complete separation of data points is eliminating the variable that is causing the problem (Allison 1999). Unfortunately, removing variables failed to eliminate quasi-complete separation of data points. Neither the full nor the reduced model is suitable for analysis.

Killed NU ALO results for the field, ABT, ASEC, and CAC are based on 268 killed NU ALO challenges at various concentrations.

The full killed NU ALO identification model which is depicted in tables 5-29 and 5-30 includes an intercept, the natural log of the concentration, indicator parameters for the JBPDSs, and indicator parameters for the chambers. Regrettably, there was quasi-complete separation of data points in this model and hence, the maximum likelihood estimate may not exist. This model is not suited for analysis with logistic regression.

Table 5-29 Killed NU ALO CAC-ASEC-ABT-Field Full Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	143.968	109.320
SC	147.551	152.322
-2 Log L	141.968	85.320
R-Square 0.1918	Max-rescaled R-Square 0.4638	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	56.6473	11	<.0001
Score	61.4553	11	<.0001
Wald	29.7446	11	0.0017

Table 5-30 Killed NU ALO CAC-ASEC-ABT-Field Full Model Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Chi-Square	Pr > ChiSq
Intercept	1	998.0	0.0015	0.9688
Natural Log of Concentration	1	0.2235	13.4089	0.0003
FIELD Indicator	1	1.4005	15.2323	<.0001
ABT Indicator	1	0.9694	6.0195	0.0141
CAC Indicator	1	0.4789	12.2385	0.0005
JBPDS2 Indicator	1	0.4406	2.1846	0.1394
JBPDS3 Indicator	0	.	.	.
JBPDS4 Indicator	1	0.6385	0.3505	0.5538
JBPDS5 Indicator	0	.	.	.
JBPDS6 Indicator	1	437.0	0.0004	0.9849
JBPDS7 Indicator	1	440.1	0.0003	0.9851
JBPDS8 Indicator	1	459.1	0.0002	0.9881
JBPDS10 Indicator	1	458.7	0.0003	0.9857
JBPDS11 Indicator	1	435.9	0.0003	0.9859
JBPDS12 Indicator	0	.	.	.

A method commonly used to develop useful models with quasi-complete separation of data points is eliminating the variable that is causing the problem (Allison 1999). The indicator parameters that identify the JBPDS are the cause of the quasi-complete separation of data points. These indicator parameters were included in the

model to control for variation between different JBPDS. As a result of correcting for quasi-complete separation of data points, the analysis of the chamber effect on identification performance is based on a simpler model which is limited to an intercept, the natural log of the concentration, and indicator parameters for the different chambers. The simpler model is described below.

The JBPDS NU ALO identification model fit statistics for the reduced CAC, ASEC, ABT, and field model are summarized in table 5-31. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-Square of 0.39 indicates that there is minimal predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 10.9283 with 8 degrees of freedom which produces a p-value of 0.2058. The deviance goodness of fit statistic is 75.3141 with 131 degrees of freedom and a p-value of unity. Neither goodness of fit test is statistically significant which suggests that the model is not a bad fit.

Table 5-31 NU ALO CAC-ASEC-ABT-Field Reduced Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	143.968	105.205
SC	147.551	123.123
-2 Log L	141.968	95.205
R-Square 0.1612	Max-rescaled R-Square 0.3898	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	46.7623	4	<.0001
Score	51.8556	4	<.0001
Wald	29.3888	4	<.0001

The intercept and the natural log of concentration both contribute to this model (p-values of 0.0004 and <0.0001 respectively). As can be seen in table 5-32, killed NU ALO identification performance in the field is statistically different from killed NU ALO identification performance in the other chambers (p-values=0.0034). The difference in the shift parameters between field and CAC is 1.4072.

Table 5-32 Killed NU ALO CAC-ASEC-ABT-Field Reduced Model Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	0.7849	12.5868	0.0004
Natural Log of Concentration	1	0.1799	15.4310	<.0001
FIELD Indicator	1	0.9059	8.5683	0.0034
ABT Indicator	1	0.6867	7.1516	0.0075
CAC Indicator	1	0.3490	12.7169	0.0004

Denatured XR identification results for the field, ABT, ASEC, and CAC are based on 119 denatured XR challenges at various concentrations.

The full denatured XR identification model which is depicted in tables 5-33 and 5-34 includes an intercept, the natural log of the concentration, indicator parameters for the JBPDSs, and indicator parameters for the chambers. Regrettably, there was qasi-complete separation of data points in this model and hence, the maximum likelihood estimate may not exist. This model is not suited for analysis.

Table 5-33 Denatured XR CAC-ASEC-ABT-Field Full Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	160.789	95.356
SC	163.568	125.927
-2 Log L	158.789	73.356
R-Square 0.5122	Max-rescaled R-Square 0.6953	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	85.4328	10	<.0001
Score	57.3734	10	<.0001
Wald	19.4780	10	0.0346

Table 5-34 Denatured XR CAC-ASEC-ABT-Field Full Model Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	533.8	0.0015	0.9686
Natural Log of Concentration	1	1.0865	16.2710	<.0001
FIELD Indicator	1	152.7	0.0031	0.9556
ABT Indicator	1	0.6460	6.5604	0.0104
CAC Indicator	1	0.5122	2.4934	0.1143
JBPDS2 Indicator	1	0.5469	2.1921	0.1387
JBPDS3 Indicator	0	.	.	.
JBPDS4 Indicator	1	0.4282	0.2053	0.6505
JBPDS5 Indicator	0	.	.	.
JBPDS7 Indicator	1	198.6	0.0000	0.9994
JBPDS8 Indicator	1	198.6	0.0000	0.9994
JBPDS9 Indicator	1	223.9	0.0000	0.9986
JBPDS11 Indicator	1	198.6	0.0000	0.9994
JBPDS12 Indicator	0	.	.	.

A method commonly used to develop useful models with quasi-complete separation of data points is eliminating the variable that is causing the problem (Allison 1999). Unfortunately, removing variables failed to eliminate quasi-complete separation of data points. Neither the full nor the reduced model is suitable for analysis with logistic regression.

Comparison of the Intercept and the Live Indicator Estimates

In the logistic models from chapters 3 through 6 the intercept shift parameter α and the live indicator shift parameters α_i are additive as seen in the following equation:

$$P(\text{Detect}|\text{Concentration}=x) = e^{(\alpha+\alpha_i+\beta x)} \ / \ (1+ e^{(\alpha+\alpha_i+\beta x)})$$

Figure 5-1 depicts the relative magnitude of intercept shift parameter α and the live indicator shift parameters α_i for each chamber for BG and killed BG from the previous chapter. The range of the intercept shift parameter α as one move across chambers is an order of magnitude greater than the range of live indicator shift parameters α_i. Hence, it appears that although the chamber effect has a profound effect on modeling detection performance, the relative magnitude and impact of the shift parameter is much less.

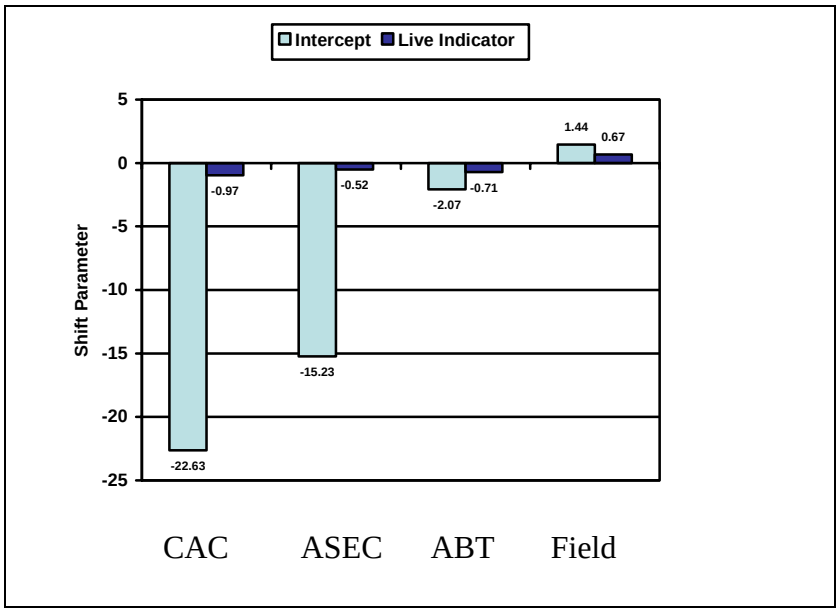


Figure 5-1 Comparison of the Intercept and the Live Indicator Estimates.

Chapter 6 Agent Simulant Models

This chapter will focus on models that could be used to predict detector performance in the field when challenged with biological warfare agent. From a test and evaluation perspective, the input parameters that would be available for that model are detector performance when challenged with either live agent or killed ALO in the CAC, and the detector performance when challenged with killed ALO in the field. Specifically this chapter will provide results and discussion for the following hypothesis.

Hypothesis 5:

- H_o: JBPDS performance can be predicted with a model based on logistic regression.
- H_a: JBPDS performance cannot be predicted with a model based on logistic regression.

Because it is important to compare a predictive model to actual performance, the modeling effort will be evaluated using BG as a surrogate. Live BG simulant will be the surrogate for live biological warfare agent. Killed BG will be the surrogate for killed ALO. The models to predict detector performance when challenged with live BG (the surrogate for live agent) in the field will be developed using killed BG (the surrogate for killed ALO) test results from both the CAC and the field as well as live BG (the surrogate for live agent) test results from the CAC. The predictive models can then be compared to

detector performance when actually challenged with live BG (the surrogate for live agent) in the field. Using this protocol, the following predictive models were developed:

- A heuristic logistic model using the method described in Holman, Russell, and Jennings (2004) based on Live BG from the CAC, and Killed BG from both the CAC and field,
- A heuristic logistic model using the method described in Holman, Russell, and Jennings (2004) based on Live BG from the CAC, and Killed BG from both the CAC and ABT,
- A heuristic logistic model extending the method described in Holman, Russell, and Jennings (2004) to include a chamber effects parameter and based on Live BG from the CAC, and Killed BG from both the CAC and field, and
- A classic logistic regression model based on Live BG from the CAC, and Killed BG from both the CAC and field

None of the heuristic logistic models were acceptable when compared to live BG detector performance field data. The Pearson goodness of fit chi square value exceeds eight billion for each of the three heuristic logistic models. Clearly and unequivocally these models are of poor fit ($p\text{-value}<0.0001$) and the null hypothesis is rejected.

On the other hand the classic logistic regression model based on live BG from the CAC, and killed BG from both the CAC and field, describe the data almost as well as a classic logistic regression model based on live BG from the field. This is a profound modeling success. However, because of the great variability in field data, the modeling process, for LE detection and identification, NU detection, and N detection offers little if

any advantage to using the ALOs as a direct proxy for detector performance against actual agent. The use of ALOs as simulants is discussed in chapter 4. In addition, because the identification properties of killed NU ALO and killed N ALO are so unlike there respective agents, the modeling process can not be applied to either NU or N. On the other hand, the modeling process has some utility for predicting both detection and identification performance of XR.

Unexplained Variability

There is much greater unexplained variability in field data than in CAC data. The CAC is a pristine environment. Temperature, humidity, and wind, are controlled in the CAC. The CAC agent and simulant aerosol clouds are homogeneous in concentration. All air entering or leaving the CAC is filtered by High Efficiency Particulate Air (HEPA) filters. The only particles present in the CAC are the simulant or agent under test. Operators and maintainers are highly skilled personnel. In the field, temperature and humidity are uncontrolled and are constantly changing. Wind in the field varies both with time and dimensional location. Winds a few meters apart can differ as much as 180^{o} difference in direction. Field simulant aerosol cloud is heterogeneous in concentration. There is also a vast array of different and unknown particles in air which vary in concentration and type over time. These particles include dirt, fungus, naturally occurring bacteria, pollen, insect parts, and other organic and inorganic debris. In field tests, systems are operated and maintained by soldiers. There is substantial variability in knowledge, skill and motivation from one soldier to another. The max-rescaled R Square can be roughly interpreted as the percentage of variability explained in the model. Figure 6-1 depicts max-rescaled R Square for the simple model that predicts detection

performance based on concentration of live BG for field and CAC releases. Clearly there is more unexplained variability in the field than in the CAC. Modeling detector performance in the field is difficult because of this unexplained variability.

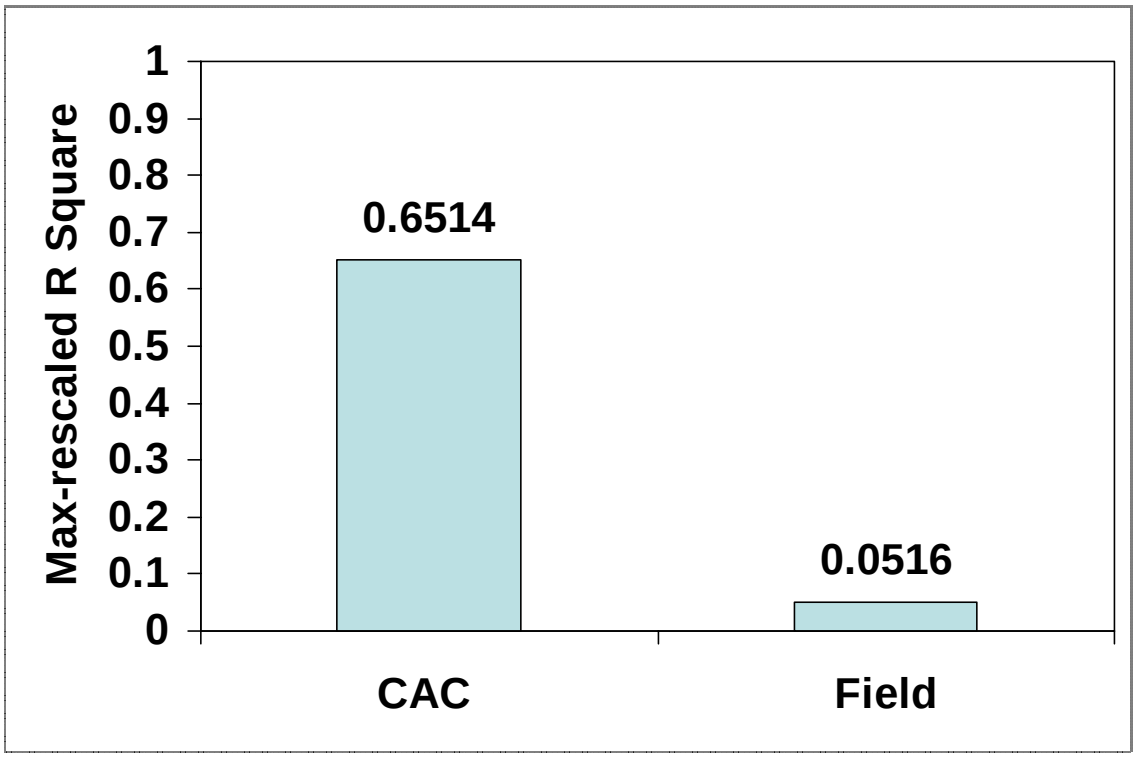


Figure 6-1 CAC and Field Max-rescaled R Square. There is greater unexplained variability in field data than in CAC data

BG Field Detection Comparative Baseline

The heuristic logistic regression detection models derived in this chapter will seek to predict live BG detection in the field. The adequacy of the heuristic logistic regression detection models will be determined by a comparison of those heuristic models to both field live BG detection data and to the logistics regression that describes detection

performance of the JBPDS when challenged with live BG simulant. This section will describe the field live BG detection performance data and logistic regression model.

Live BG Field detection results are based on 108 BG challenges at various concentrations of which 83 were detected and 28 were not detected.

The JBPDS BG detection model fit statistics for the field are summarized in table 6-1. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-Square of 0.05 indicates that there is minimal predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 16.2278 with 8 degrees of freedom which produces a p-value of 0.0392. The deviance goodness of fit statistic is 58.3575 with 47 degrees of freedom and a p-value of 0.1238. The Hosmer and Lemeshow goodness of fit test is statistically significant which suggests a poor model fit. The deviance goodness of fit test is not statistically significant which suggests that the model is not a bad fit.

Table 6-1 Live BG Field Detection Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	125.613	123.744
SC	128.295	129.108
-2 Log L	123.613	119.744
R-Square 0.0352	Max-rescaled R-Square 0.0516	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF Pr > ChiSq
Likelihood Ratio	3.8691	1 0.0492
Score	3.6880	1 0.0548
Wald	3.5582	1 0.0593

The intercept does not contribute statistically to this model (p-value=0.2665). The natural log of concentration is only marginally statistically significant (p-value=0.0593). The shape parameter β for the live BG field logistic regression is 0.2161. The α shift parameter for the live BG field logistic regression is 0.4222. These statistics are summarized in table 6-2.

Table 6-2 Live BG Field Detection Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	0.4222	0.3800	1.2348	0.2665
Natural Log of Concentration	1	0.2161	0.1146	3.5582	0.0593

The relationship between detection and concentration is depicted in figure 6-2.

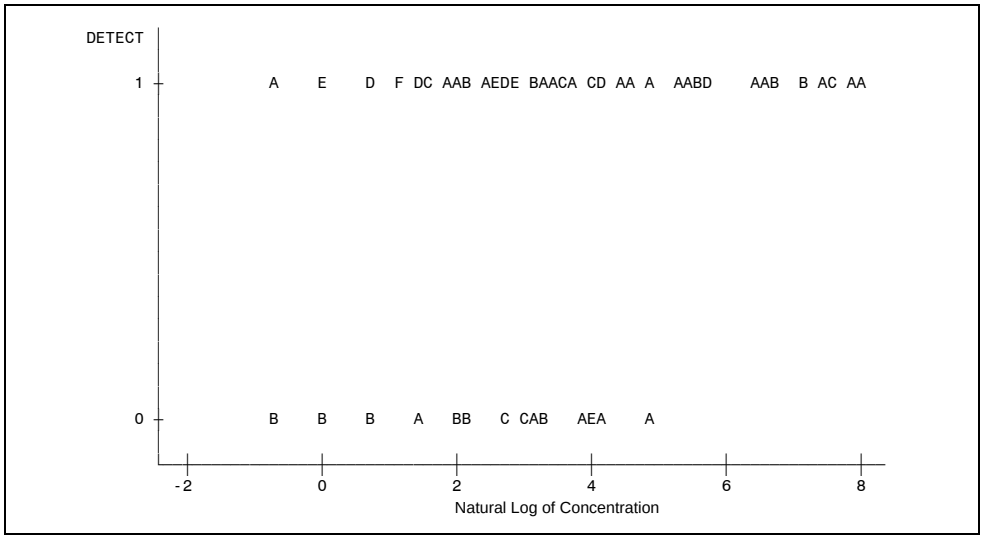


Figure 6-2 Live BG Field Detection Performance. A positive detection is plotted as Detect = 1. A negative detection is plotted Detect = 0. "A" represents 1 data value, "B" represents 2 data values, "C" represents 3 data values, and so on.

For comparison to residuals from heuristic models in subsequent sections, a residual plot is provided for the base line in figure 6-3.

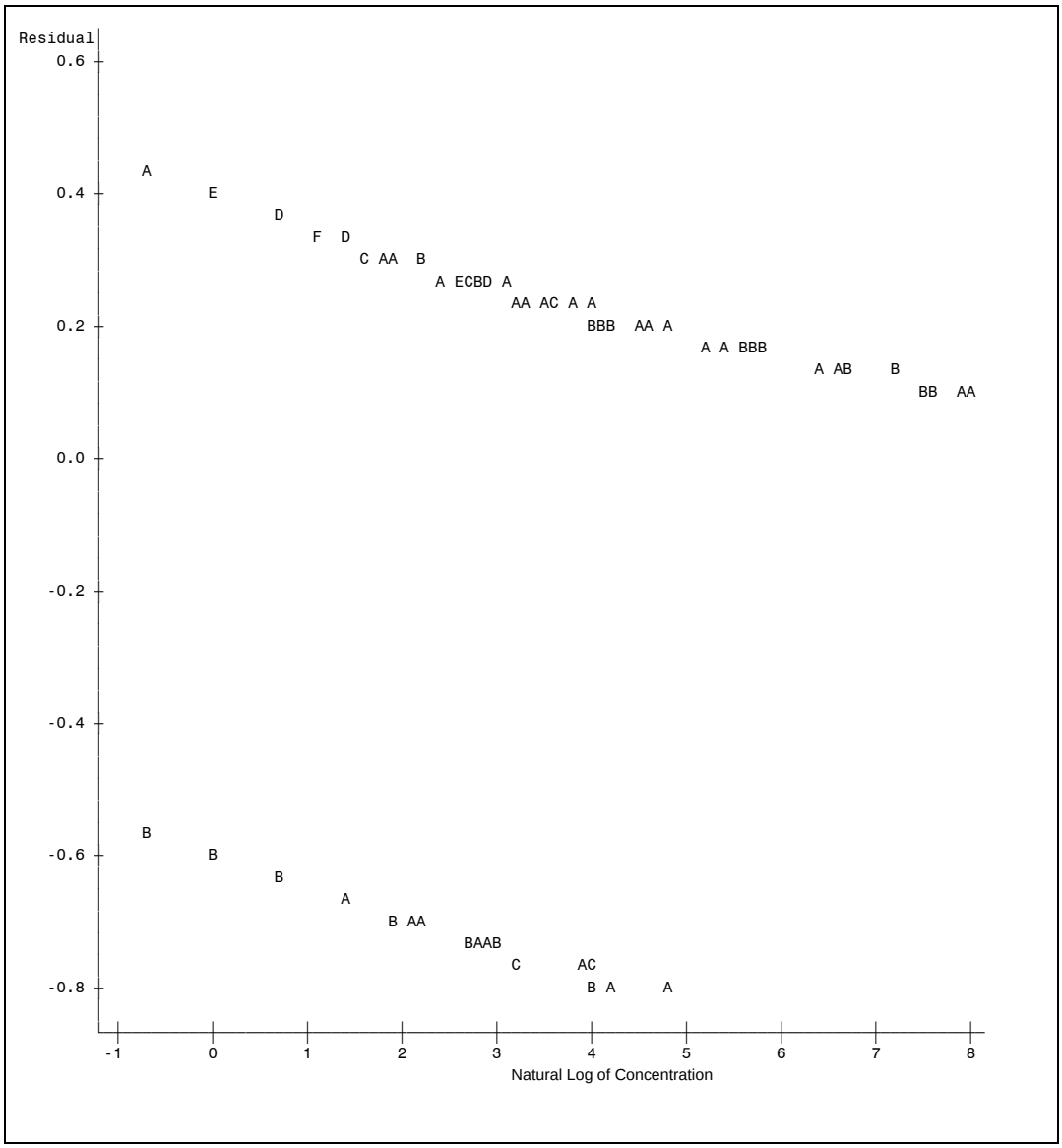


Figure 6-3 Live BG Field Detection Residual Plot. The residual is equal to the difference between the actual detection performance (0=no detection, 1=detection) and the predicted probability of detection based on a logistic regression analysis of live BG field detection. "A" represents 1 data value, "B" represents 2 data values, "C" represents 3 data values, and so on.

Detection Heuristic Based on CAC and Field Data

This section will develop the shape parameter β and the shift parameter α to be used in the heuristic logistic regression. The source of the shape parameter β will be killed BG field detection trials. The source of the shift parameter α will be live BG CAC trials.

Killed BG Field detection results are based on 80 BG challenges at various concentrations of which 73 were detected and 7 were not detected.

The JBPDS BG detection model fit statistics for the field are summarized in table 6-3. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-Square of 0.12 indicates that there is minimal predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 7.4814 with 7 degrees of freedom which produces a p-value of 0.3805. The deviance goodness of fit statistic is 28.3928 with 35 degrees of freedom and a p-value of 0.7777. Neither goodness of fit tests are statistically significant which suggests that the model is not a bad fit.

Table 6-3 Killed BG Field Detection Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	49.474	46.990
SC	51.856	51.754
-2 Log L	47.474	42.990
R-Square 0.0545	Max-rescaled R-Square 0.1218	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	4.4849	1	0.0342
Score	4.0125	1	0.0452
Wald	3.5523	1	0.0595

The intercept and the natural log of concentration both contribute to this model as depicted in table 6-4 (p-values of 0.0004 and 0.0595 respectively).

The shape parameter β from the killed BG field logistic regression becomes the shape parameter β for the heuristic logistic regression. As can be seen from table 6-2 the value for β is -0.5926. The negative shape parameter β is counter intuitive. It implies that as concentration increases that detection performance decreases. Clearly, something other than concentration is influencing detection performance.

Table 6-4 Killed BG Field Detection Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	4.1484	1.1794	12.3725	0.0004
Natural Log of Concentration	1	-0.5926	0.3144	3.5523	0.0595

The relationship between detection and concentration is depicted in figure 6-4. There plot supports the observation that as concentration increased, detection performance decreases.

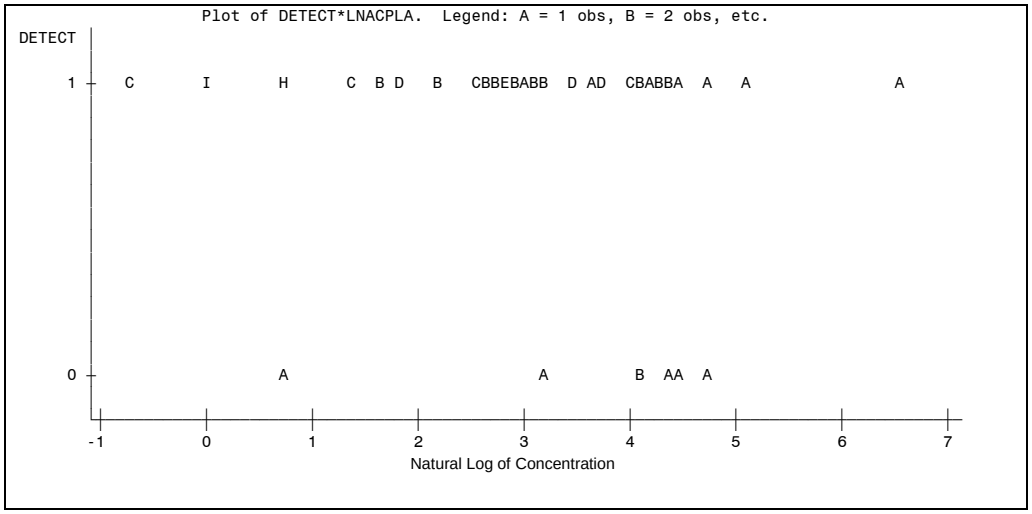


Figure 6-4 Killed BG Field Detection Performance. A positive detection is plotted as Detect = 1. A negative detection is plotted Detect = 0. "A" represents 1 data value, "B" represents 2 data values, "C" represents 3 data values, and so on.

Live BG CAC detection results are based on 18 live BG challenges at various concentrations of which 18 were detected and 18 were not detected.

The JBPDS live BG detection model fit statistics for the field are summarized in table 6-5. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-Square of 0.65 indicates that there is predictive value in this model, but not as much as some of the others.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 11.817 with 7 degrees of freedom which produces a p-value of 0.1067. The deviance goodness of fit

statistic is 12.8835 with 15 degrees of freedom and a p-value of 0.6113. Neither goodness of fit tests are statistically significant which suggests that the model is not a bad fit.

Table 6-5 Live BG CAC Detection Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	26.953	16.883
SC	27.844	18.664
-2 Log L	24.953	12.883
R-Square 0.4886	Max-rescaled R-Square 0.6514	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	12.0698	1	0.0005
Score	9.7515	1	0.0018
Wald	5.4061	1	0.0201

The intercept and the natural log of concentration both contribute to this model as depicted in table 6-6 (p-values of 0.0209 and 0.0201 respectively).

The shift parameter α from the live BG CAC logistic regression becomes the shift parameter α for the heuristic logistic regression. As can be seen from table 6-6 the value for α is -15.8454. This is on a natural log scale.

Table 6-6 Live BG CAC Detection Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	-15.8454	6.8583	5.3379	0.0209
Natural Log of Concentration	1	4.5850	1.9720	5.4061	0.0201

Hence, the heuristic logistic regression model based on the shift parameter α from live BG in the CAC and the shape parameter β from the killed BG in the field is:

$$P(\text{Detect}|\text{Concentration}=x) = e^{(-15.8454-0.5926x)} / (1+ e^{(-15.8454-0.5926x)})$$

Goodness of Fit: Heuristic Based on CAC and Field

The goodness of fit of the of the BG detection heuristic logistic regression based on the shift parameter α from live BG in the CAC and the shape parameter β from the killed BG in the field will be analyzed in this section.

A residual plot depicts the error of the prediction over the range of the independent variable. For the BG detection heuristic logistic regression the error of the prediction is the difference between the actual detection performance (0=no detection, 1=detection) and the predicted probability of detection based on the heuristic logistic regression model. This model is based on the shift parameter α from live BG in the CAC and the shape parameter β from the killed BG in the field. So that the BG detection heuristic logistic regression residual plot could be compared to the baseline, a plot was also constructed for the logistic regression of live BG field detection. The residual results are summarized in figures 6-3 and 6-5. As would be expected, there is less error in the residual plot based on the logistic regression of live BG field detection (fig 6-3). However, it is clear that the distribution of errors from the heuristic logistic regression is undesirable. The heuristic logistic regression is correctly predicting missed detections. However, detections are being underestimated by unity or some number close to unity.

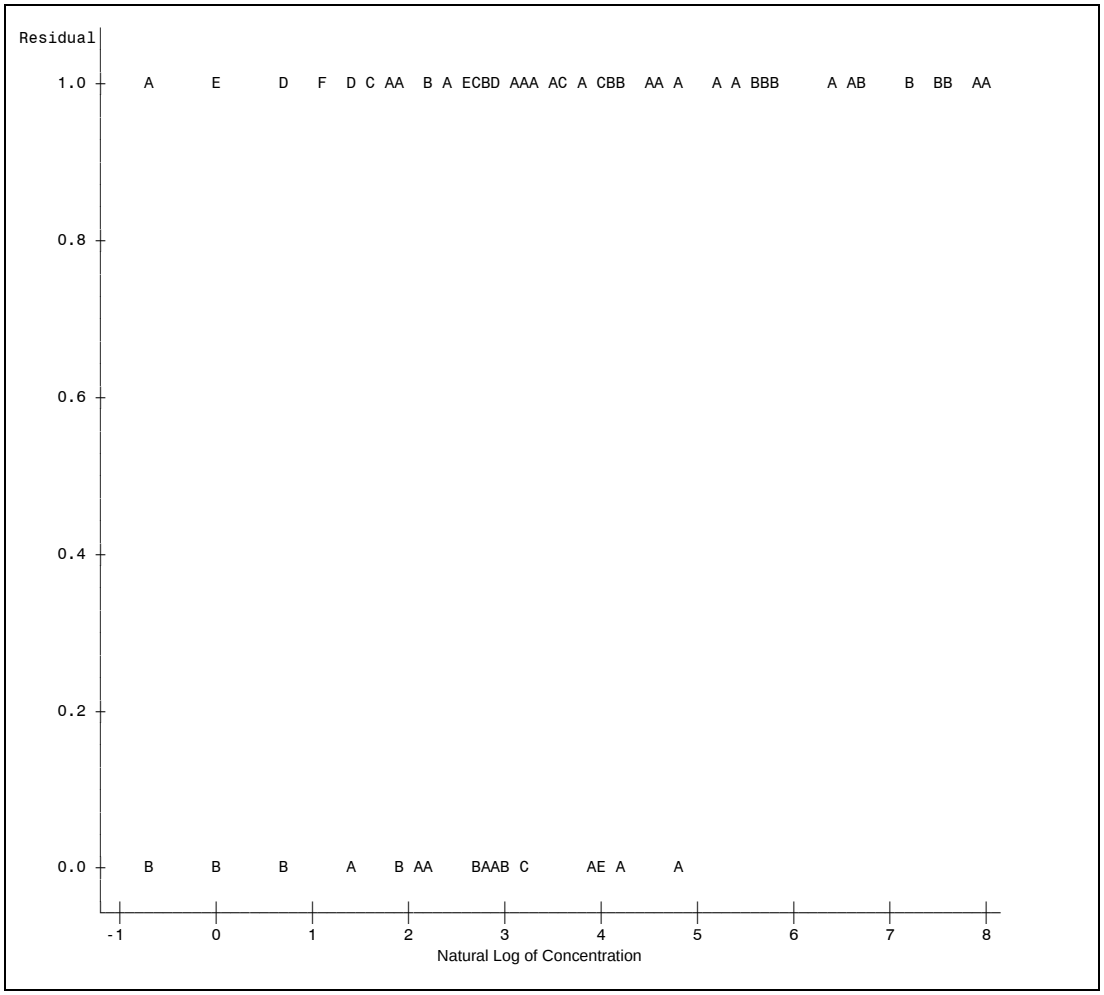


Figure 6-5 Heuristic BG Field Detection Residual Plot. This heuristic is based on the shift parameter α from live BG in the CAC and the shape parameter β from the killed BG in the field. The residual is equal to the difference between the actual detection performance (0=no detection, 1=detection) and the predicted probability of detection based on a logistic regression analysis of live BG field detection. "A" represents 1 data value, "B" represents 2 data values, "C" represents 3 data values, and so on.

Based on the difference in predicted performance plotted over concentration, the live BG field detection heuristic logistic regression, based on the shift parameter α from the live BG in the CAC and the shape parameter β from the killed BG in the field is not an acceptable substitute for the actual logistic regression derived from JBPDS using live BG. The difference in JBPDS predicted performance between the logistic regression

model and the heuristic regression model is depicted in Figure 6-6. The points in this figure are derived by subtracting the predicted JBPDS detection performance using the heuristic logistic regression from the predicted JBPDS detection performance using the base line logistic regression for live BG field detection. Clearly the two models agree only at very low concentrations (less than 1 ACPLA).

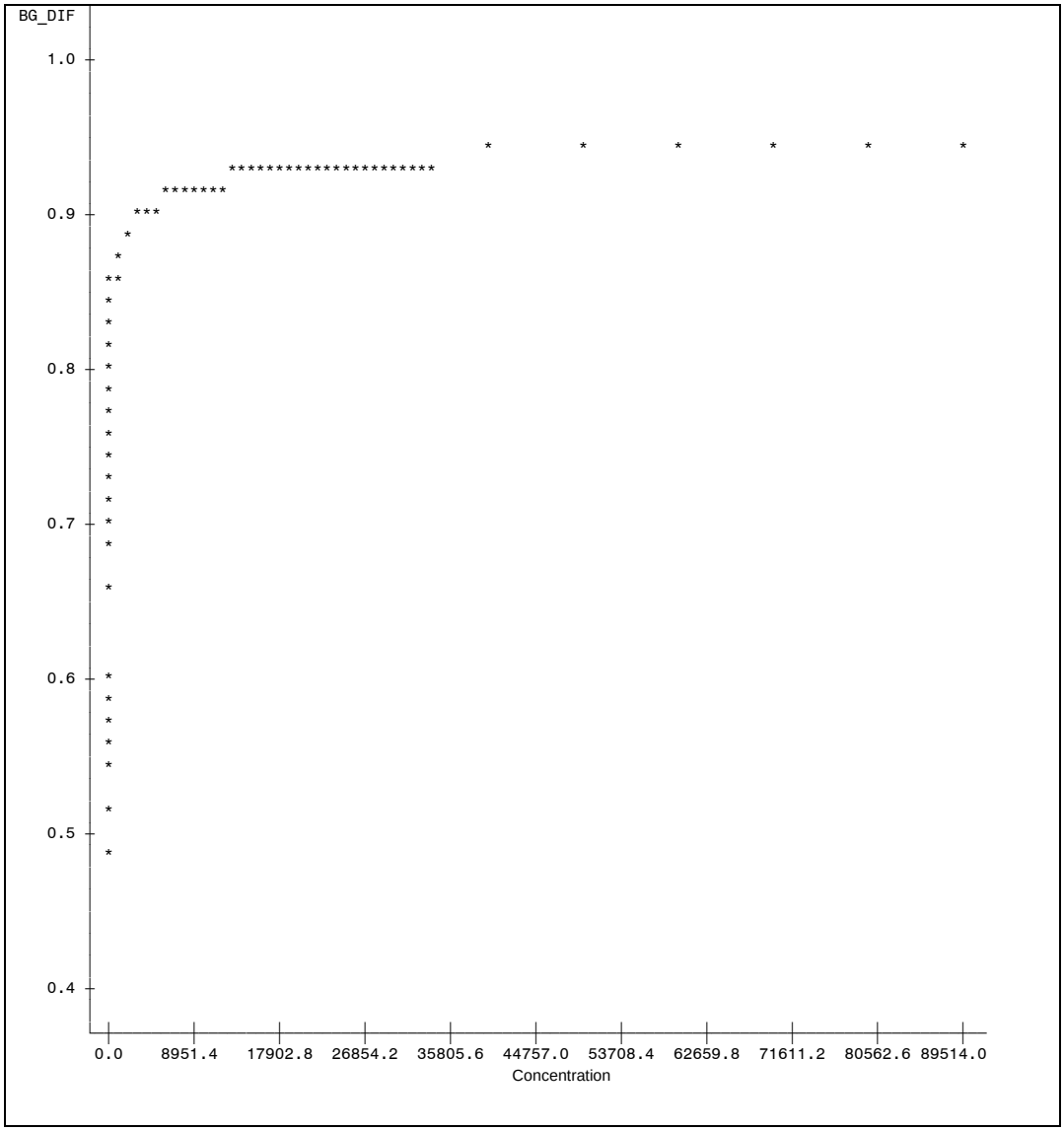
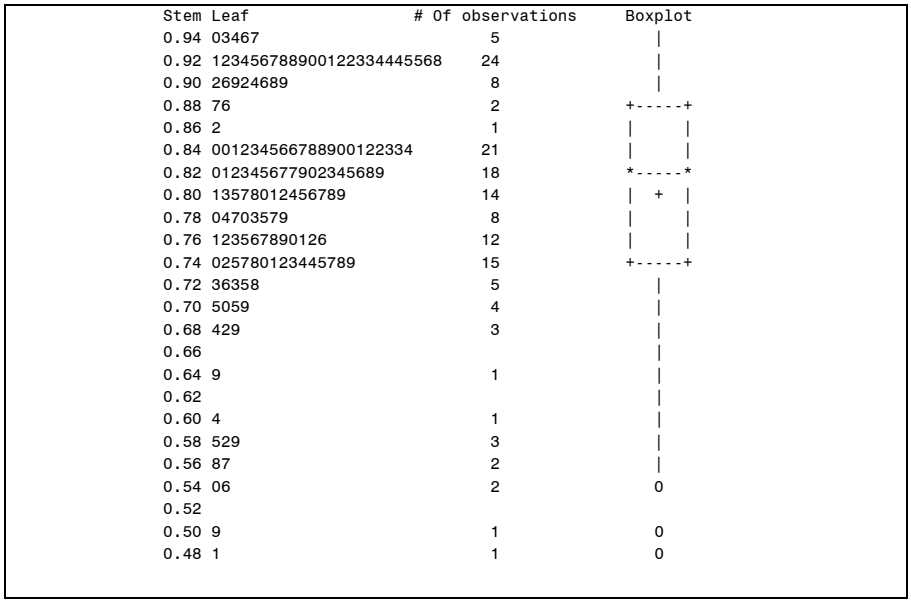


Figure 6-6 Difference in JBPDS Predicted Performance Between the Logistic Regression Model and the Heuristic Model. This heuristic Model is based on the shift parameter α from the live BG in the CAC and the shape parameter β from the killed BG in the ABT.

Based on the distribution of differences in performance, the live BG field detection heuristic logistic regression, based on the shift parameter α from the live BG in the CAC and the shape parameter β from the killed BG in the field is not an acceptable substitute for the actual logistic regression derived from JBPDS using live BG. As

depicted in figure 6-7 the maximum, seventy-fifth percentile, median, twenty-fifth percentile and minimum differences in detection performance between the two models predicted probability of detection are: 0.947, 0.895, 0.823, 0.759, and 0.481 respectively.



Stem	Leaf	# Of observations	Boxplot
0.94	03467	5	
0.92	12345678900122334445568	24	
0.90	26924689	8	
0.88	76	2	
0.86	2	1	
0.84	001234566788900122334	21	
0.82	012345677902345689	18	
0.80	13578012456789	14	
0.78	04703579	8	
0.76	123567890126	12	
0.74	025780123445789	15	
0.72	36358	5	
0.70	5059	4	
0.68	429	3	
0.66			
0.64	9	1	
0.62			
0.60	4	1	
0.58	529	3	
0.56	87	2	
0.54	06	2	
0.52			
0.50	9	1	
0.48	1	1	

Figure 6-7 Stem Leaf and Box Plot of the Difference in JBPDS Predicted Performance Between Logistic Regression Model with the Heuristic Regression Model. This heuristic model is based on the shift parameter α from live BG in the CAC and the shape parameter β from the killed BG in the field.

The Pearson goodness of fit chi square value is 8,719,999,902 with 7 degrees of freedom. Clearly and unequivocally this model is a poor fit (p-value<0.0001).

Detection Heuristic Based on CAC and ABT

Clearly, because of the inconsistency in the sign of the shape parameter β, killed BG field detection performance can not be the source for the shape parameter β. The next best source for the shape parameter β is killed BG from the ABT.

Killed BG ABT detection results are based on 130 BG challenges at various concentrations of which 52 were detected and 78 were not detected.

The JBPDS BG detection model fit statistics for the field are summarized in table 6-7. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-Square of 0.4604 indicates that there is some predictive value in this model, but not as much as some of the others.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 4.5585 with 7 degrees of freedom which produces a p-value of 0.7137. The deviance goodness of fit statistic is 96.9532 with 60 degrees of freedom and a p-value of 0.0018. The Hosmer and Lemeshow goodness of fit test is not statistically significant which suggests a good fit. However, deviance goodness of fit statistic is statistically significant which suggests a poor fit.

Table 6-7 Killed BG ABT Detection Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	176.983	124.860
SC	179.851	130.595
-2 Log L	174.983	120.860
R-Square 0.3405	Max-rescaled R-Square 0.4604	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	54.1230	1	<.0001
Score	46.9680	1	<.0001
Wald	31.6951	1	<.0001

The intercept and the natural log of concentration both contribute to this model as depicted in table 6-6 (p-values <0.0001 and <0.0001 respectively).

The shape parameter β from the killed BG ABT logistic regression becomes the shape parameter β for the heuristic logistic regression. As can be seen from table 6-8 the value for β is 0.8736.

Table 6-8 Killed BG ABT Detection Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	-2.6425	0.4435	35.4957	<.0001
Natural Log of Concentration	1	0.8736	0.1552	31.6951	<.0001

The relationship between detection and concentration is depicted in figure 6-8.

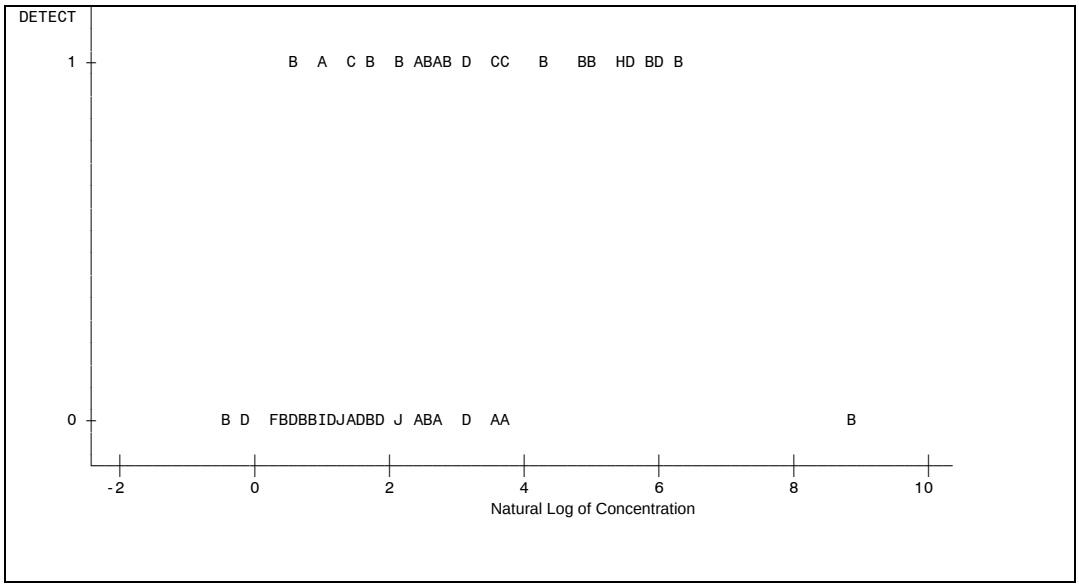


Figure 6-8 Killed BG Field Detection Performance. A positive detection is plotted as Detect = 1. A negative detection is plotted Detect = 0. "A" represents 1 data value, "B" represents 2 data values, "C" represents 3 data values, and so on.

Live BG CAC detection results are based on 18 live BG challenges at various concentrations of which 18 were detected and 18 were not detected.

The JBPDS live BG detection model fit statistics for the field are summarized in table 6-5. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-Square of 0.65 indicates that there is predictive value in this model, but not as much as some of the others.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 11.817 with 7 degrees of freedom which produces a p-value of 0.1067. The deviance goodness of fit statistic is 12.8835 with 15 degrees of freedom and a p-value of 0.6113. Neither goodness of fit tests are statistically significant which suggests that the model is not a bad fit.

The intercept and the natural log of concentration both contribute to this model as depicted in table 6-6 (p-values of 0.0209 and 0.0201 respectively).

The shift parameter α from the live BG CAC logistic regression becomes the shift parameter α for the heuristic logistic regression. As can be seen from table 6-6 the value for α is -15.8454.

Hence, the heuristic logistic regression model based on the shift parameter α from live BG in the CAC and the shape parameter β from the killed BG in the ABT is:

$$P(\text{Detect}|\text{Concentration}=x) = e^{(-15.8454+0.8736x)} / (1+ e^{(-15.8454+0.8736x)})$$

Goodness of Fit: Heuristic Based on CAC and ABT

The goodness of fit of the of the BG detection heuristic logistic regression based on the shift parameter α from live BG in the CAC and the shape parameter β from the killed BG in the ABT will be analyzed in this section.

A residual plot depicts the error of the prediction over the range of the independent variable. For the BG detection heuristic logistic regression the error of the prediction is the difference between the actual detection performance (0=no detection, 1=detection) and the predicted probability of detection based on the heuristic logistic regression analysis. So that the BG detection heuristic logistic regression residual plot could be compared to the baseline, a plot was also constructed for the logistic regression of live BG field detection. The residual results are summarized in figures 6-3 and 6-9. As would be expected, there is less error in the residual plot based on the logistic regression of live BG field detection (fig 6-3). However, it is clear that the distribution of errors from the heuristic logistic regression is undesirable. The heuristic logistic regression is correctly predicting missed detections. However, detections are being underestimated by unity or some number close to unity.

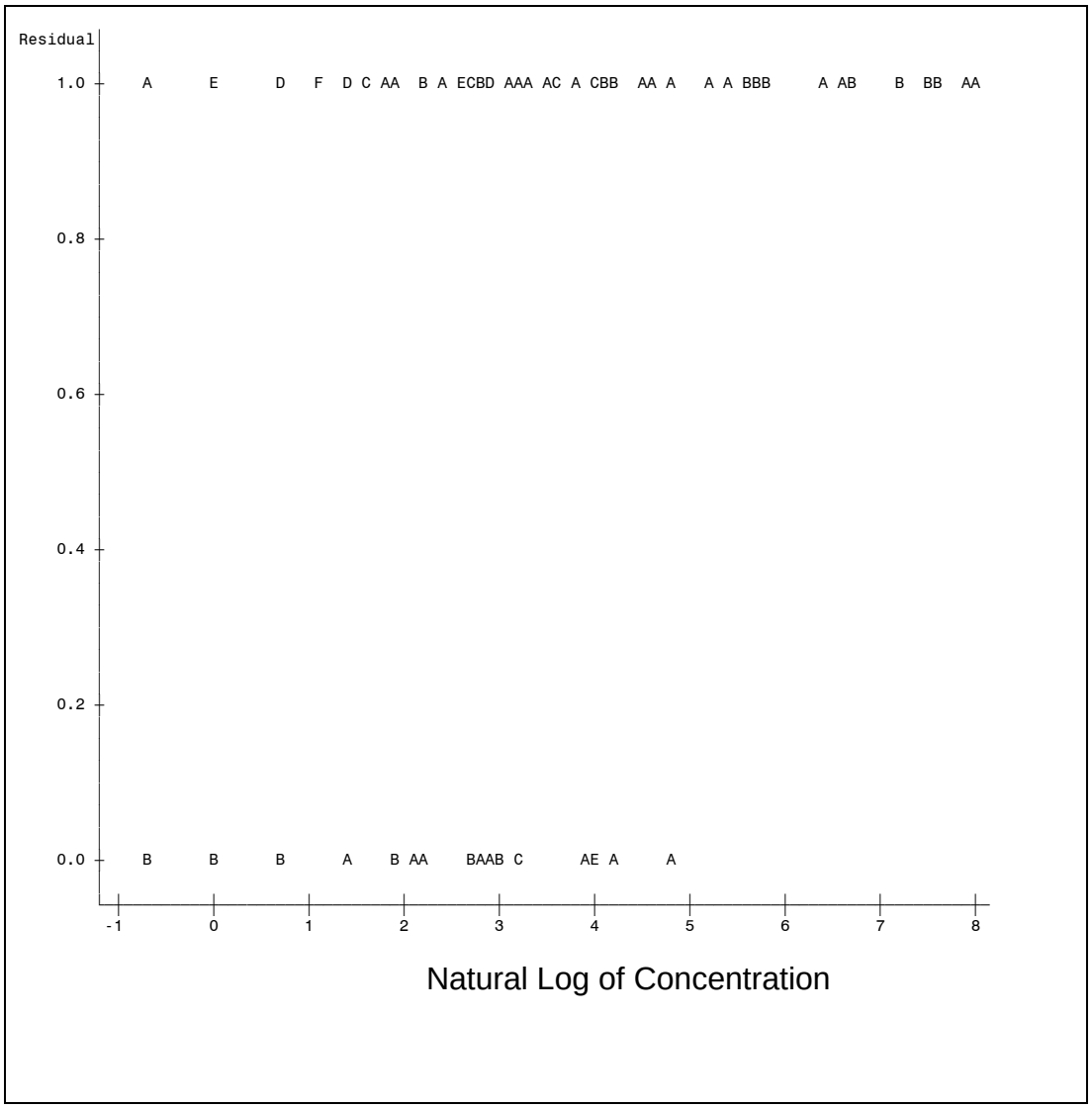


Figure 6-9 Live BG Field Heuristic Logistic Regression Detection Residual Plot. The residual is equal to the difference between the actual detection performance (0=no detection, 1=detection) and the predicted probability of detection based on a heuristic logistic regression analysis of live BG field detection. "A" represents 1 data value, "B" represents 2 data values, "C" represents 3 data values, and so on.

Based on the difference in predicted performance plotted over concentration, the live BG field detection heuristic logistic regression, based on the shift parameter α from the live BG in the CAC and the shape parameter β from the killed BG in the ABT is not

an acceptable substitute for the actual logistic regression derived from JBPDS using live BG. The difference in JBPDS predicted performance between the logistic regression model and the heuristic regression model is depicted in Figure 6-10. The points in this figure are derived by subtracting the predicted JBPDS detection performance using the heuristic logistic regression from the predicted JBPDS detection performance using the base line logistic regression for live BG field detection. Clearly the two models agree only at very low concentrations (less than 1 ACPLA).

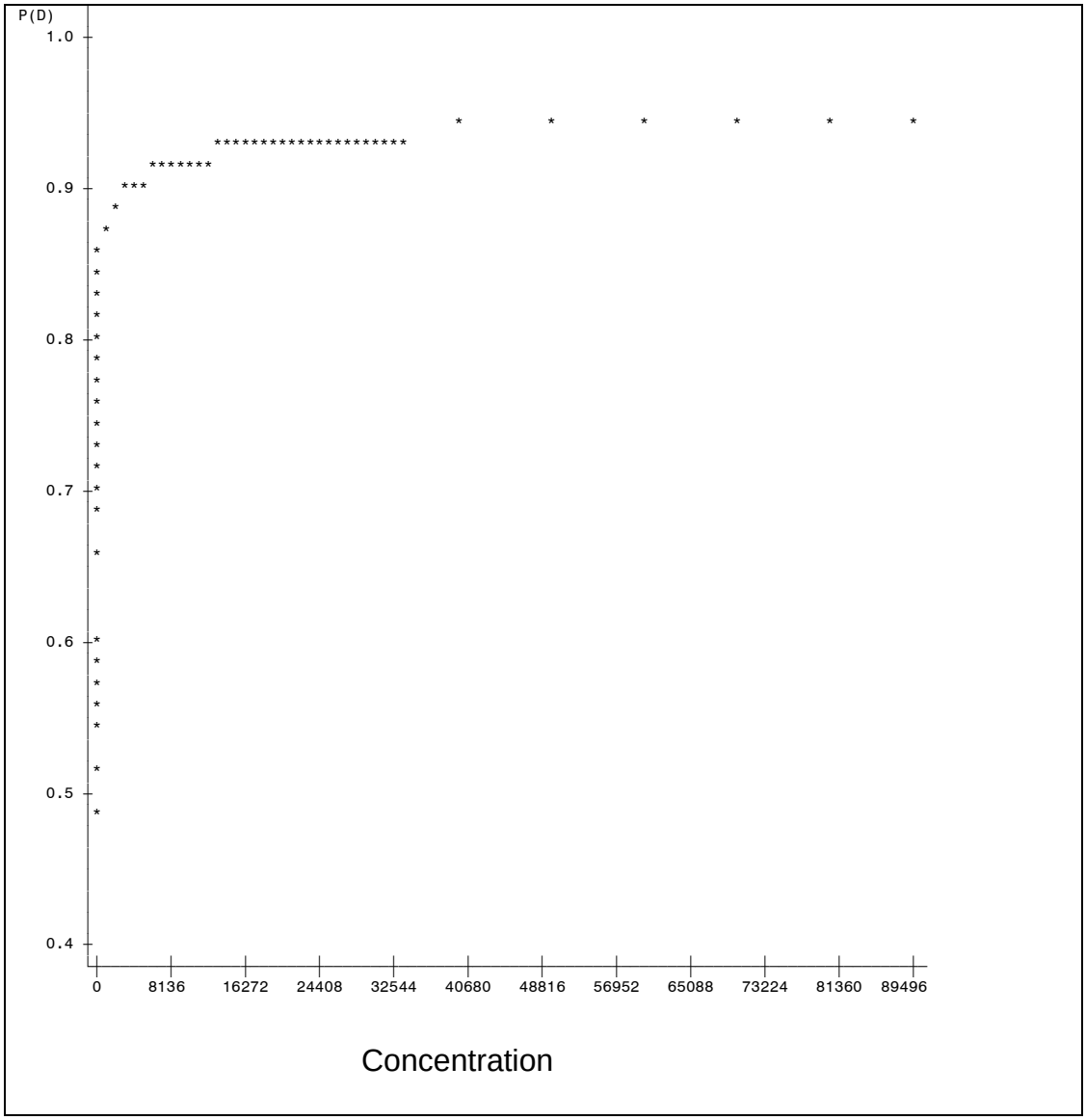
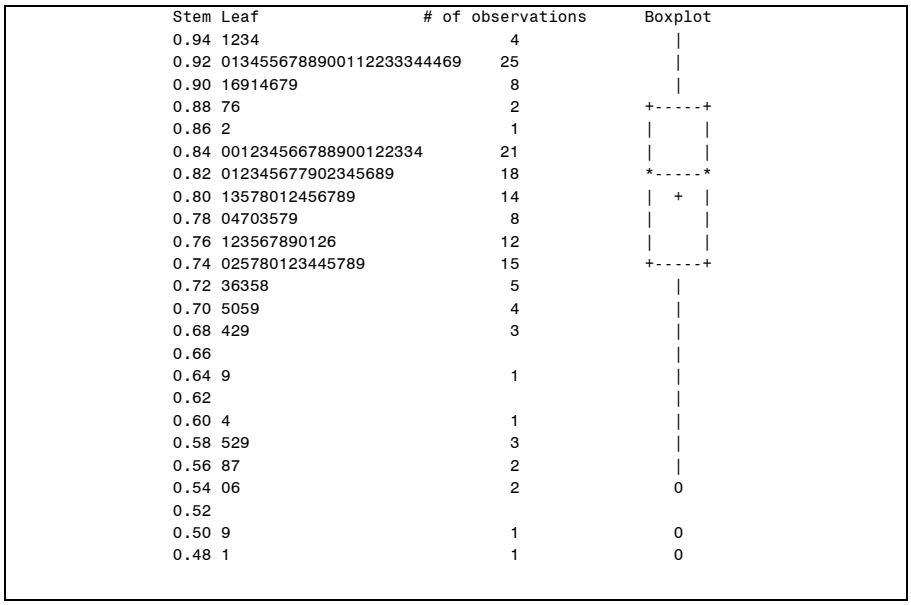


Figure 6-10 Difference in JBPDS Predicted Performance Between the Logistic Regression Model and the Heuristic Model. This heuristic Model is based on the shift parameter α from the live BG in the CAC and the shape parameter β from the killed BG in the ABT.

Based on the distribution of differences in performance, the live BG field detection heuristic logistic regression, based on the shift parameter α from the live BG in the CAC and the shape parameter β from the killed BG in the ABT is not an acceptable

substitute for the actual logistic regression derived from JBPDS using live BG. As depicted in figure 6-11 the maximum, seventy-fifth percentile, median, twenty-fifth percentile and minimum differences in detection performance between the two models predicted probability of detection are: 0.944, 0.896, 0.823, 0.759, and 0.481 respectively.



Stem	Leaf	# of observations	Boxplot
0.94	1234	4	
0.92	013455678890011223334446 9	25	
0.90	16914679	8	
0.88	76	2	+-----+
0.86	2	1	| |
0.84	001234566788900122334	21	| |
0.82	012345677902345689	18	*-----*
0.80	13578012456789	14	| + |
0.78	04703579	8	| |
0.76	123567890126	12	| |
0.74	025780123445789	15	+-----+
0.72	36358	5	
0.70	5059	4	
0.68	429	3	
0.66			
0.64	9	1	
0.62			
0.60	4	1	
0.58	529	3	
0.56	87	2	
0.54	06	2	0
0.52			
0.50	9	1	0
0.48	1	1	0

Figure 6-11 Stem Leaf and Box Plot of the Difference in JBPDS Predicted Performance Between Logistic Regression Model with the Heuristic Regression Model. This heuristic model is based on the shift parameter α from live BG in the CAC and the shape parameter β from the killed BG in the ABT.

The Pearson goodness of fit chi square value is 8,719,999,902 with 7 degrees of freedom. Clearly and unequivocally this model is a poor fit (p-value<0.0001).

Using the β from the killed BG in the ABT in the heuristic logistic model offered no advantage to using the β from the killed BG in the field. Both heuristic models are of poor fit and not acceptable.

Detection Heuristic Based on CAC and Field Data with a Chamber Effect

As documented and discussed in chapter 5, there is a chamber shift parameter α. Incorporating a chamber shift parameter α into the heuristic logistic model could potentially improve the predictive performance of the model. From table 5-4 the difference between the field effect α and CAC effect α is 1.8408. Hence, when adjusting for the chamber effect the new heuristic model becomes:

$$P(\text{Detect}|\text{Concentration}=x) = e^{(-15.8454+1.8408-0.5926x)} / (1+ e^{(-15.8454+1.8408-0.5926x)})$$

Goodness of Fit: Heuristic Based on CAC and Field with a Chamber Effect

A residual plot depicts the error of the prediction over the range of the independent variable. For the BG detection heuristic logistic regression the error of the prediction is the difference between the actual detection performance (0=no detection, 1=detection) and the predicted probability of detection based on the heuristic logistic regression model. This model is based on the shift parameter α from live BG in the CAC and the shape parameter β from the killed BG in the field and an adjustment for chamber effect. So that the BG detection heuristic logistic regression residual plot could be compared to the baseline, a plot was also constructed for the logistic regression of live BG field detection. The residual results are summarized in figures 6-3 and 6-12. As would be expected, there is less error in the residual plot based on the logistic regression of live BG field detection (fig 6-3). However, it is clear that the distribution of errors from the heuristic logistic regression is undesirable. The heuristic logistic regression is correctly predicting missed detections. However, detections are being underestimated by unity or some number close to unity.

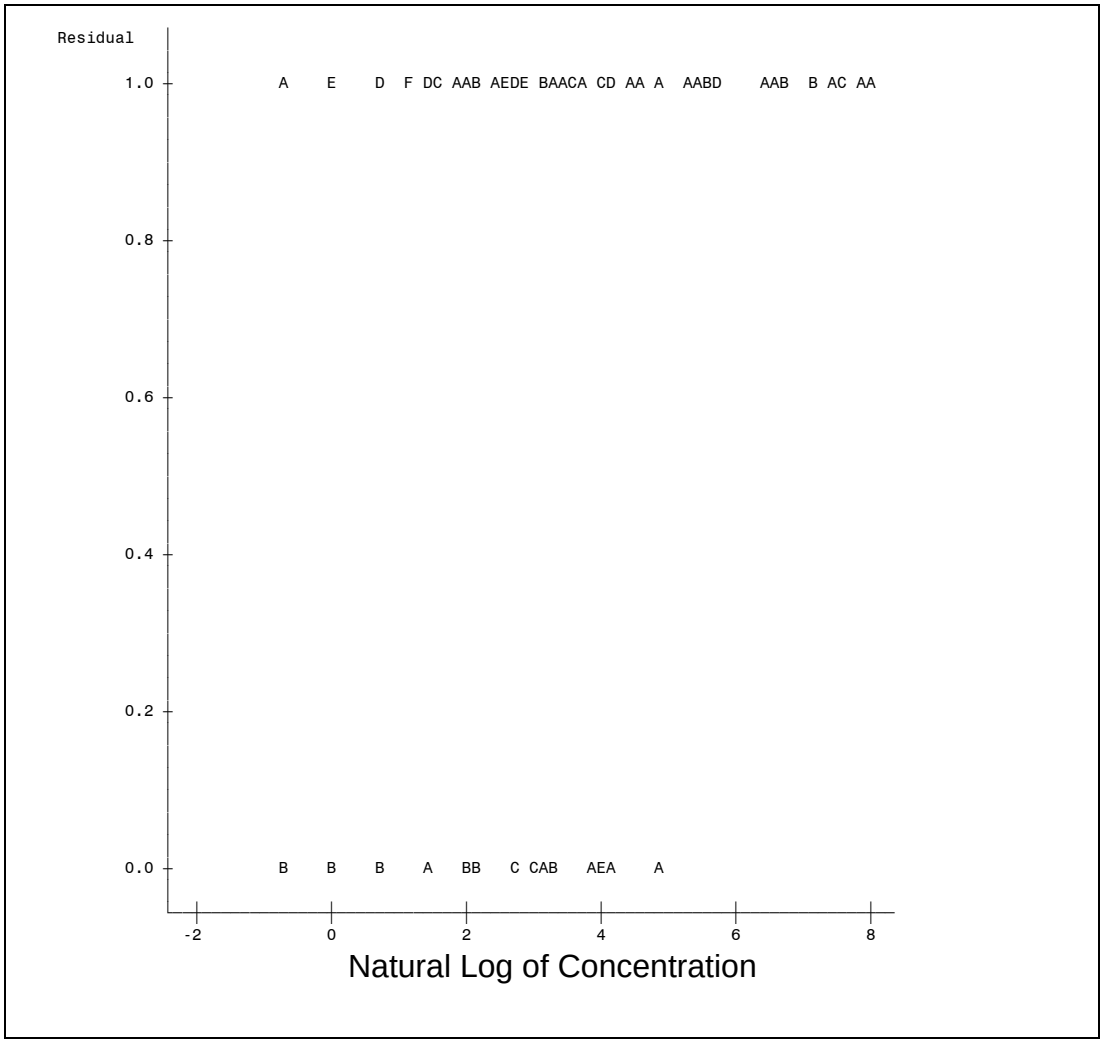


Figure 6-12 Heuristic BG Field Detection Residual Plot. This heuristic is based on the shift parameter α from live BG in the CAC and the shape parameter β from the killed BG in the field and an adjustment for chamber effect. The residual is equal to the difference between the actual detection performance (0=no detection, 1=detection) and the predicted probability of detection based on a logistic regression analysis of live BG field detection. "A" represents 1 data value, "B" represents 2 data values, "C" represents 3 data values, and so on.

Based on the difference in predicted performance plotted over concentration, the live BG field detection heuristic logistic regression, based on the shift parameter α from the live BG in the CAC, the shape parameter β from the killed BG in the field, and an

adjustment for chamber effect is not an acceptable substitute for the actual logistic regression derived from JBPDS using live BG. The difference in JBPDS predicted performance between the logistic regression model and the heuristic regression model is depicted in Figure 6-13. The points in this figure are derived by subtracting the predicted JBPDS detection performance using the heuristic logistic regression from the predicted JBPDS detection performance using the base line logistic regression for live BG field detection. Clearly the two models agree only at very low concentrations (less than 1 ACPLA).

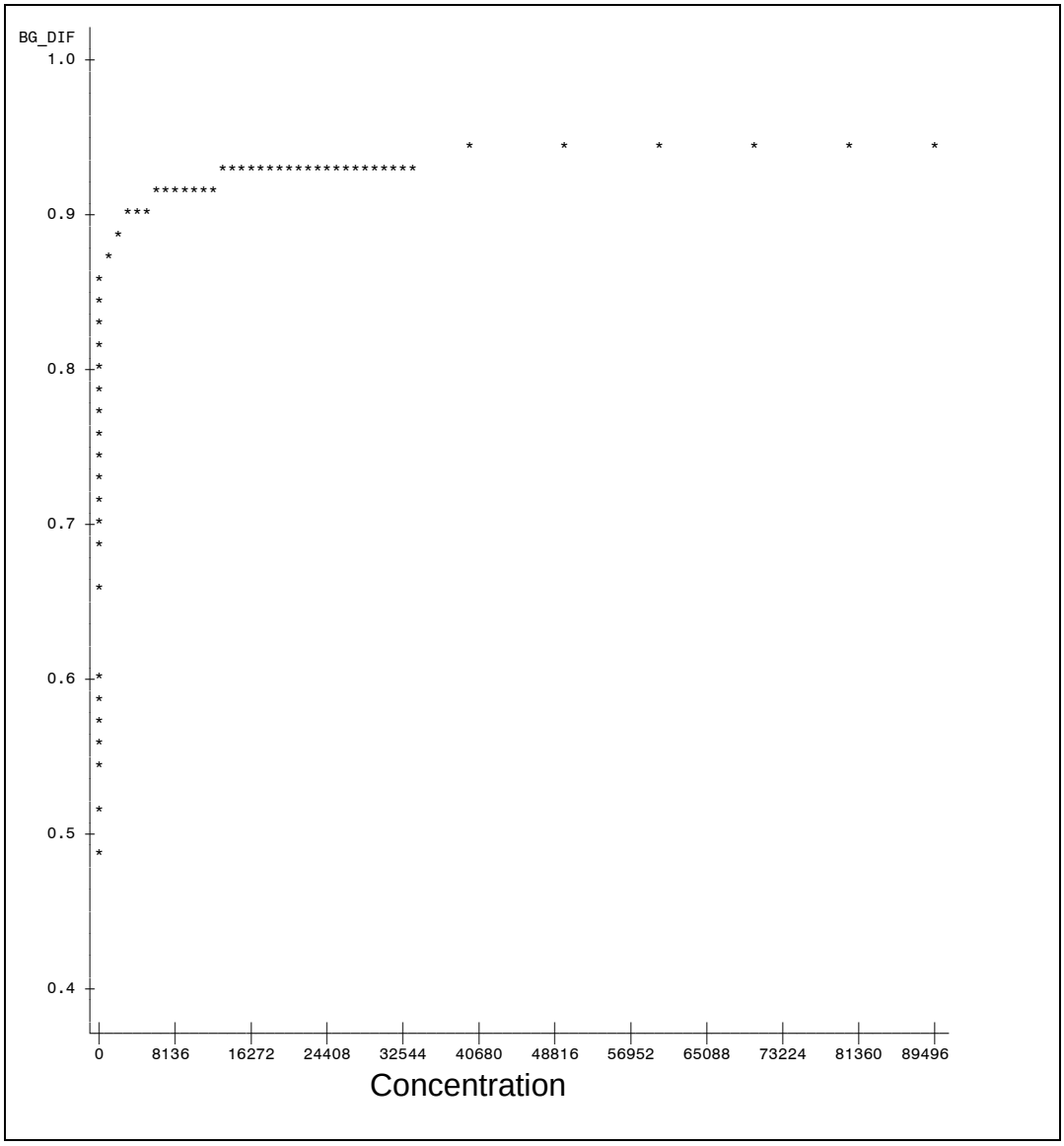
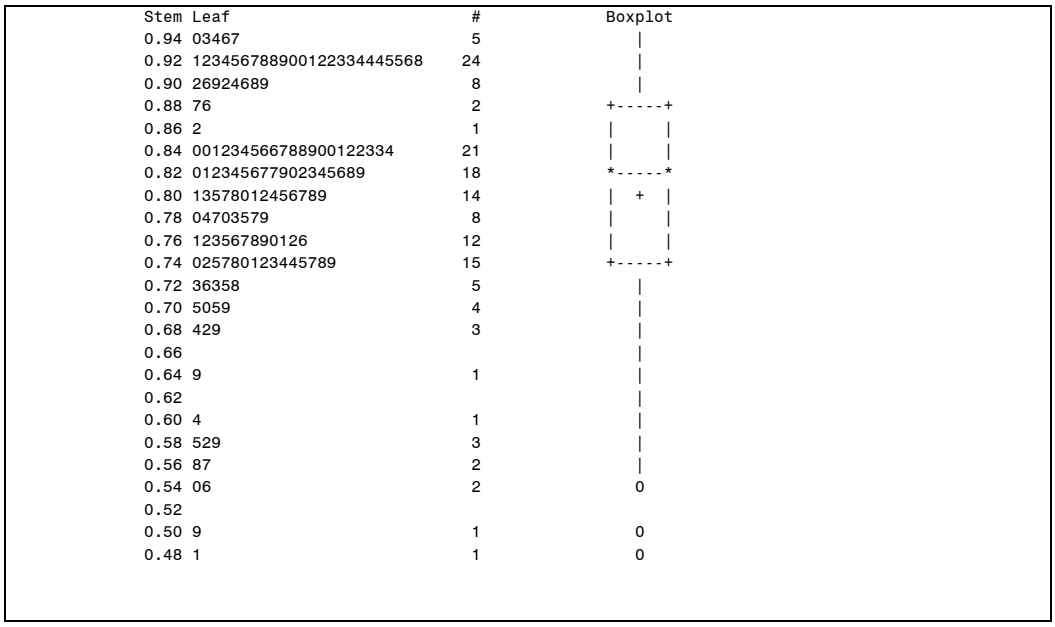


Figure 6-13 Difference in JBPDS Predicted Performance Between the Logistic Regression Model and the Heuristic Model. This heuristic Model is based on the shift parameter α from the live BG in the CAC and the shape parameter β from the killed BG in the ABT and an adjustment for chamber effect.

Based on the distribution of differences in performance, the live BG field detection heuristic logistic regression, based on the shift parameter α from the live BG in the CAC, the shape parameter β from the killed BG in the field, and an adjustment for

chamber effect is not an acceptable substitute for the actual logistic regression derived from JBPDS using live BG. As depicted in figure 6-7 the maximum, seventy-fifth percentile, median, twenty-fifth percentile and minimum differences in detection performance between the two models predicted probability of detection are: 0.947, 0.895, 0.823, 0.759, and 0.481 respectively.



Stem	Leaf	#	Boxplot
0.94	03467	5	
0.92	123456788900122334445568	24	
0.90	26924689	8	
0.88	76	2	
0.86	2	1	
0.84	001234566788900122334	21	
0.82	012345677902345689	18	
0.80	13578012456789	14	
0.78	04703579	8	
0.76	123567890126	12	
0.74	025780123445789	15	
0.72	36358	5	
0.70	5059	4	
0.68	429	3	
0.66			
0.64	9	1	
0.62			
0.60	4	1	
0.58	529	3	
0.56	87	2	
0.54	06	2	
0.52			
0.50	9	1	
0.48	1	1	

Figure 6-14 Stem Leaf and Box Plot of the Difference in JBPDS Predicted Performance Between Logistic Regression Model with the Heuristic Regression Model. This heuristic model is based on the shift parameter α from live BG in the CAC, the shape parameter β from the killed BG in the field and an adjustment for chamber effect.

The Pearson goodness of fit chi square value is 8,719,999,902 with 7 degrees of freedom. Clearly and unequivocally this model is a poor fit (p-value<0.0001).

Classic Logistic Regression Detection Model

Since the heuristic detection models described above are so extremely poor, a model will be constructed using classical logistic regression techniques and detection

data from the CAC on live and killed BG and detection data from the field on killed BG. This model will then be used to predict performance of live BG in the field.

Live and killed BG CAC and killed BG field detection results are based on 123 BG challenges at various concentrations of which 98 were detected and 25 were not detected.

The JBPDS BG detection model fit statistics for the CAC-field are summarized in table 6-9. Clearly, at least one coefficient in the model is not equal to zero. A rescaled R-Square of 0.23 indicates that there is minimal predictive value in this model.

The Hosmer and Lemeshow goodness of fit test Chi-Square value is 32.2438 with 9 degrees of freedom which produces a p-value of 0.0002. The deviance goodness of fit statistic is 89.3367 with 73 degrees of freedom and a p-value of 0.0939. Both goodness of fit tests, especially the Hosmer and Lemeshow indicate that this is a poor fit.

Table 6-9 Live and Killed BG CAC and Killed BG Field Detection Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	126.200	111.933
SC	129.012	123.182
-2 Log L	124.200	103.933
R-Square 0.1519	Max-rescaled R-Square 0.2390	
Testing Global Null Hypothesis: All coefficients=0		
Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	20.2664	3	0.0001
Score	20.9442	3	0.0001

The intercept, natural log of concentration, and killed indicator are not statistically significant in this model (p-values of 0.3552, 0.2832, and 0.5329 respectively). As can

be seen in table 6-10, the Chamber indicator, which is identified as CAC indicator has a statically significant impact on this model.

Table 6-10 Live and Killed BG CAC and Killed BG Field Detection Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	0.5720	0.6187	0.8548	0.3552
Natural Log of Concentration	1	0.2114	0.1970	1.1514	0.2832
CAC Indicator	1	-1.0995	0.3627	9.1870	0.0024
Killed Indicator	1	0.2024	0.3246	0.3889	0.5329

From table 6-10 the model for live BG detection in the field is:

$$P(\text{Detect}|\text{Concentration}=x) = e^{(0.5720+0.2114x)} / (1+ e^{(0.5720+0.2114x)})$$

Goodness of Fit: Classic Logistic Regression Detection Model

This section will examine the model developed using classical logistic regression techniques and detection data from the CAC on live and killed BG and detection data from the field on killed BG; and determine how well that model predicts JBPDS performance against live BG in the field.

A residual plot depicts the error of the prediction over the range of the independent variable. This model is used to predict JBPDS performance when challenged with live BG. It was developed using classical logistic regression techniques and detection data from the CAC on live and killed BG and detection data from the field on killed BG. The residuals for this model as depicted in figure 6-15 are as good as the

residuals for the logistic regression developed from live BG challenges in the field which are depicted in figure 6-3.

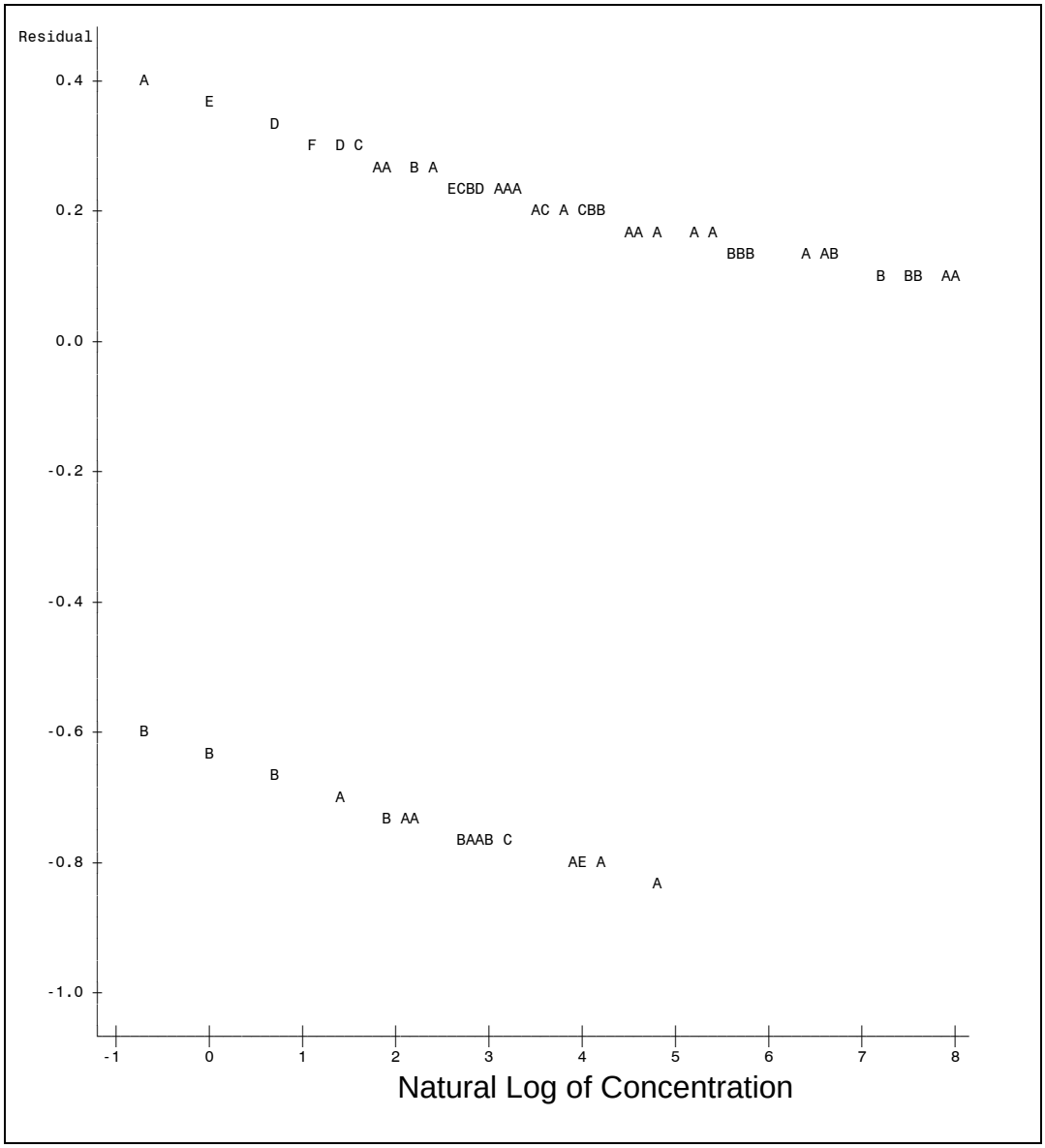


Figure 6-15 BG Field Detection Residual Plot. Model used was developed by classical logistic regression techniques and detection data from the CAC on live and killed BG and detection data from the field on killed BG. This model was then used to predict JBPDS performance in the field with live agent. The residual is equal to the difference between the actual detection performance (0=no detection, 1=detection) and the predicted probability of detection based on a logistic regression analysis of live BG field detection. "A" represents 1 data value, "B" represents 2 data values, "C" represents 3 data values, and so on.

The difference in the probability of detection based on two logistic regression models is quite small. As seen in figure 6-16, the difference never exceeds 0.0049. The two models predict quite similarly.

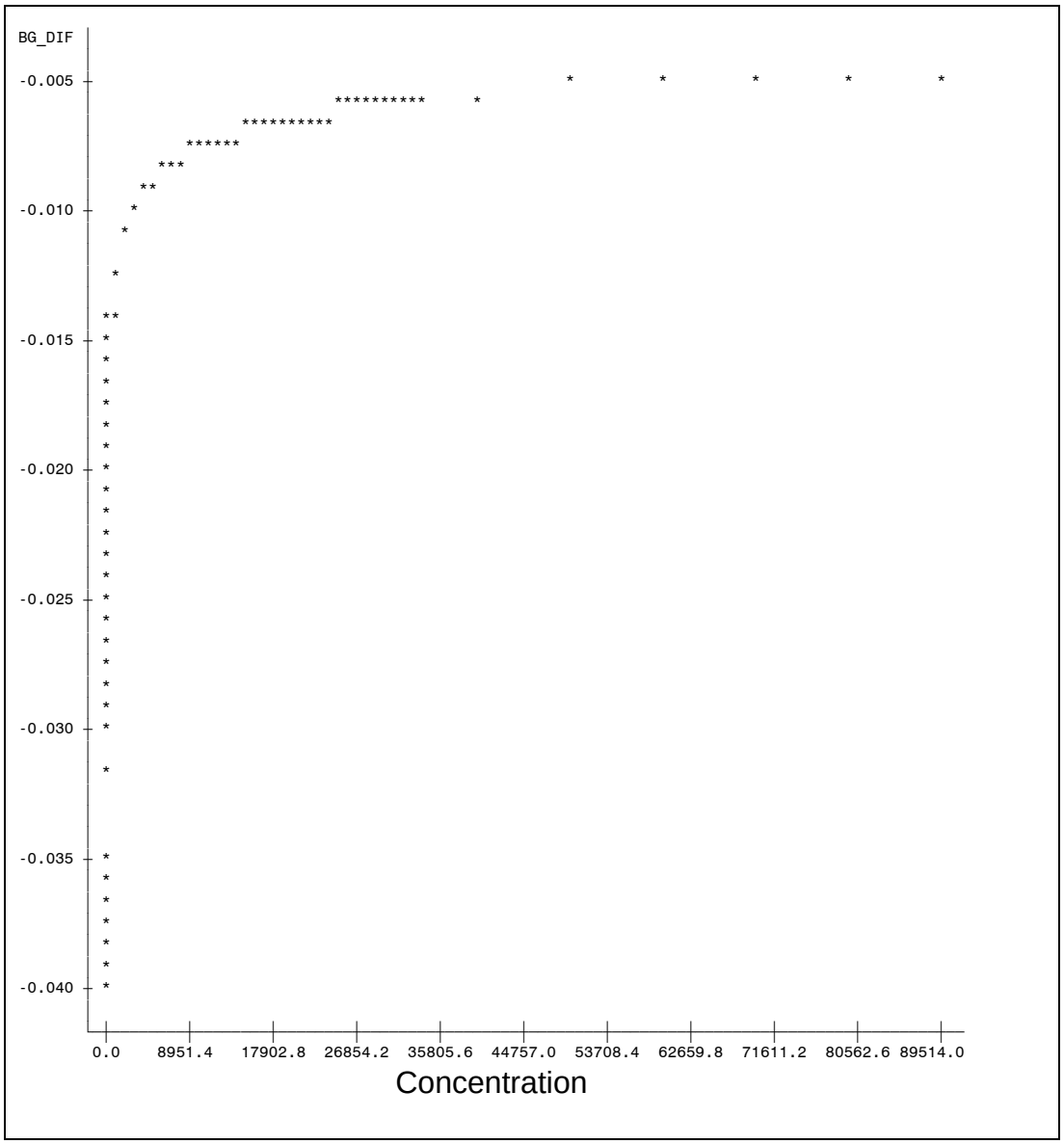
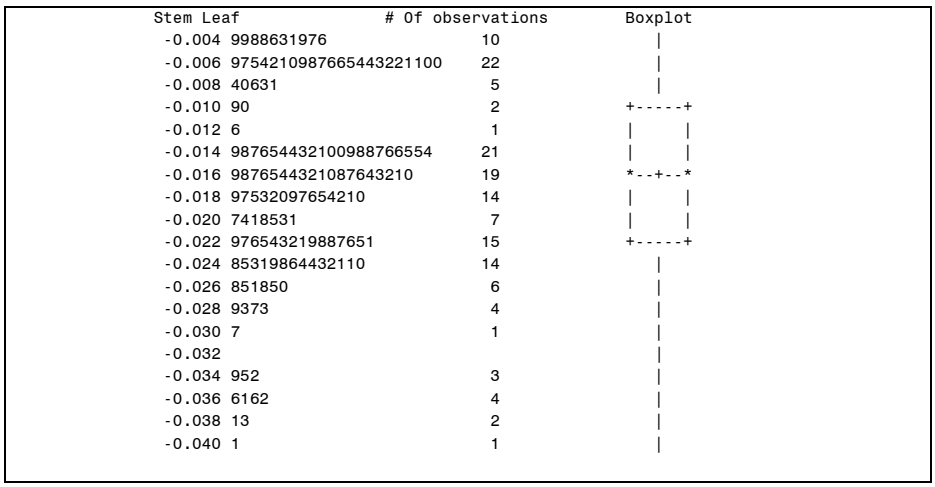


Figure 6-16 Difference in JBPDS Field BG Predicted Performance. The difference in the probability of detection based on two logistic regression models. One developed using live BG in the field. The other developed using live and killed BG in the CAC and Killed BG in the field.

Based on the distribution of differences in performance, the two models are remarkably similar in their predictions. As depicted in figure 6-17 the maximum, seventy-fifth percentile, median, twenty-fifth percentile and minimum differences in detection performance between the two models predicted probability of detection are: -0.004, -0.010, -0.018, -0.024, and -0.040 respectively.



Stem Leaf	# Of observations	Boxplot
-0.004 9988631976	10	|
-0.006 9754210987665443221100	22	|
-0.008 40631	5	|
-0.010 90	2	+-----+
-0.012 6	1	| |
-0.014 987654432100988766554	21	| |
-0.016 98765443210876432l0	19	*--+--*
-0.018 97532097654210	14	| |
-0.020 7418531	7	| |
-0.022 976543219887651	15	+-----+
-0.024 85319864432110	14	|
-0.026 851850	6	|
-0.028 9373	4	|
-0.030 7	1	|
-0.032		|
-0.034 952	3	|
-0.036 6162	4	|
-0.038 13	2	|
-0.040 1	1	|

Figure 6-17 Stem Leaf and Box Plot of the Difference in JBPDS Predicted Performance Between the two Logistic Regression Models.

The Pearson goodness of fit chi square value is 14.7929 with 7 degrees of freedom. This model is a poor fit (p-value=0.051). However, this goodness of fit is similar to what was observed for the baseline (Hosmer and Lemrshoe p-value=0.0392 and deviance p-value=0.1238).

Based upon the residual plot, and the differences in predictions of the two models, the model to predict JBPDS live BG detection performance in the field based on live and

killed BG in the CAC and killed BG in the field is as acceptable as the model that is based on live BG in the field.

Chapter 7 Conclusion

Conclusions

Detector performance in a BL3 chamber such as the CAC is not representative of detector performance in the field. In the CAC the detector is more sensitive and less variable in response than in the field. The sensitivity in the CAC may be as much as two orders of magnitude greater than its sensitivity in the field. As a stand alone estimator, detector performance in the CAC is not an acceptable predictor of biological warfare agent detector performance in the field.

Killed ALOs are acceptable detection simulants in the field to be used to predict biological warfare agent detection field performance against actual BWA. For the agents N and XR there is weak statistical difference between detection performance with the actual agent and its corresponding ALO (p-value=0.0905 and 0.0603 respectively). On the other hand, for agents LE and NU there is statistical difference between detection performance with the actual agent and its corresponding ALO (p-value=0.0437 and 0.0234 respectively). Nevertheless because of the magnitude of the concentration range over which this difference in detection performance occurs and the expected number of challenges in that range, killed LE ALO, killed NU ALO, killed N ALO, and denatured XR are acceptable simulants to be used in field tests to predict biological warfare agent detection performance. Results from JBPDS detection performance in field trials when

challenged with these killed ALOs can be used as a substitute for detector performance against the corresponding BWA.

Some but not all killed ALOs are acceptable simulants in the field to be used to predict biological warfare agent identification field performance against actual BWA. There is no statistical difference in biological warfare agent identification performance between challenges of LE and killed LE ALO (p-value=0.1562). Killed LE ALO is an acceptable identification simulant for LE. On the other hand, for agent XR and the simulant denatured XR there is statistical difference in identification performance (p-value=0.0294). However, since the expected number of field trials that would fall in the range that produces differences in performance is relatively small, denatured XR is judged to be an acceptable simulant for XR. At concentrations in which killed NU ALO was readily identified, no NU was identified. At concentrations in which N was readily identified, no killed N ALO was identified. Neither killed NU ALO nor killed N ALO are acceptable simulants for their corresponding agents. Results from JBPDS identification performance in field trials when challenged with killed LE ALO or denatured XR can be used as a substitute for identification performance against the corresponding BWA.

The simple models examined in this dissertation do not adequately explain the variability that occurs in the field. As a result of unexplained variability even the best predictive model is of minimal utility in predicting detector performance.

The best predictor of biological warfare agent detector performance is field trials with killed ALOs. Killed LE ALO and denatured XR are acceptable for both detection and identification. Killed N ALO and Killed NU ALO are acceptable for detection only.

Future Research

The objectives of this dissertation were:

1. To determine if killed or inactivated Agent-Like Organism (ALO) are good simulants for a biological point detection system that use particle fluorescence and immunoassay technology;
2. To determine if a simple model can be developed to relate detector simulant performance to detector performance with biological warfare agent and if that model is a reasonable analytical construct for use in evaluation.

For the first objective, it was determined that Killed N ALO and Killed NU ALO are not acceptable identification simulants for N and NU. There is a need to develop a better identification simulant for N and NU that can be used in open air releases. For the second objective because of unexplained variability a useful simple model could not be developed to relate detector simulant performance to detector performance with biological warfare agent. There is a need to develop a better understanding of the critical variables, beyond concentration and agent type, in predicting field detector performance and then to develop a better model to predict field detector performance.

Simulant development is a long process. There are many technical challenges to developing an acceptable simulant and many legal and regulatory challenges to obtaining authorization to release that simulant in an open air test. It took over 5 years to develop killed ALOs as simulants and obtain legal and regulatory approval to release them. However, there is a potential shortcut that could provide a useful N and NU identification simulant prior to the JBPDS full rate production decision this fall. The mechanism of JBPDS identification is an antigen and antibody reaction. During identification, the

antigen on the simulant or agent combines with the antibody on the identification strip. Antigens are primarily proteins. The structure of an antigen affects the affinity of an antibody to that antigen. The ALOs are killed by gamma. Gamma radiation alters the structure of a protein. Hence, it is possible to adjust the identification of the sensitivity of the JBPDS to the killed ALOs by changing the amount of ionizing radiation that is used to kill them. Hence, by changing the amount of ionizing radiation used to kill N ALO and NU ALO they could become useful identification simulants. The amount of ionizing radiation that the ALOs are subjected to must be sufficient to ensure their death. If the altering of the killing process for N ALO and NU ALO fails to produce useful identification simulants that can safely be used in a field test, then "bugbeads" offer an alternative. "Bugbeads" are polystyrene beads coated with the outer surface of bacteria. Their use as a simulant is discussed in chapter 2 in the section on Biological Simulants.

In the short term, to gain additional insight into understanding the nature of critical variables beyond concentration and agent type in predicting field detector performance, I have initiated two efforts. First a review of the JBPDS engineering model and second, continued data analysis of the JBPDS field data. There is a detailed engineering simulation model of JBPDS. A review of this model may provide additional insight on the importance of various environmental factors on predicting performance. The additional data analysis will be exploratory in nature and focused on causes of variability in performance. This analysis will include development of a detailed temporal and spatial aerosol cloud profile for each simulant release, and a comparison of individual system performance focusing on why some system performance response is more variable than others.

In the long term to gain a better scientific understanding of critical variables that effect field detector performance there is a need for additional basic research. One area that seems potentially fruitful is the effect of type and quantity of ambient partials in the air on detector performance.

LITERATURE CITED

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Allison, PD 1999. Logistic regression using SAS. SAS Institute and Wiley, Cary, NC, 288 pages.

Agresti, A 1996. An introduction to categorical data analysis. John Wiley & Sons, New York, 290 pages.

Banks HT, C Castillo-Chavez 2003. Bioterrorism mathematical modeling applications in homeland security. Philadelphia, Society for Industrial and Applied Mathematics. 240 p.

Battat, B 2005. Chemical and biological threat analysis. Defense Threat Reduction Agency, Joint Science Technology Office - Chemical and Biological Agent Simulant Summit Aug 10-11, 2005, Computer Science Corporation Executive Briefing Center, Falls Church, VA.

Chipman, M, CE Holman, K Finanger, J Fuller, R Jernigan, L Wollenberg, B Rodriguez, S Lepper, D Alverez, G Sierra, D Andersen, J Thermos, C Jennings, E Pierce (2001) System assessment of the Joint Biological Point Detection System. US Army ATEC Report Alexandria VA.

Christian, P, CE Holman 1995. Biological Integrated Detection System (BIDS) Test and Evaluation Report (TER). US Army ATEC Report Alexandria VA.

Clementi, S, M Stevens 2005. Simulants: Individual protection equipment. Defense Threat Reduction Agency, Joint Science Technology Office - Chemical and Biological Agent Simulant Summit Aug 10-11, 2005, Computer Science Corporation Executive Briefing Center, Falls Church, VA.

Davis JM 1987. Modelong the long-range transport of plant pathogens in the atmosphere. Ann. Rev. Phytopathol 25: 169-88.

DTRA 2007a. Simulant summit: Notes from the biological weapons working group. Defense Threat Reduction Agency, Joint Science Technology Office - Chemical and Biological Agent Simulant Summit May 8, 2007, Arlington, VA

DTRA 2007b. Simulant summit: Notes from the chemical weapons working group. Defense Threat Reduction Agency, Joint Science Technology Office - Chemical and Biological Agent Simulant Summit May 8, 2007, Arlington, VA

Farrell S, HB Halsall, WR Heineman 2005. Bacillus globigii bugbeads: a model simulant of bacterial spores. Anal Chem 77: 549-55.

Farrow, SW, JN Thermos, SL Schanaman, T Lindsey, BS Williams 2002 Abbreviated Test Report for the First Article Test of the Joint Biological Point Detection System. WDTC-TR-02-014, West Desert Test Center, Dugway, UT, 89 pages.

Fitch JP, BP Mark, W Colclasure, M Coleman, PE Coyle, HH Hill, NB Jackson, DL Jan, B Johnson, T Moshier, SN Rudnick, BI Swanson, DR Walt 2004 Review of test and evaluation methodology for biological point detectors. Washington DC, the National Academies Press. 106 p.

Ghosal D, MV Omelchenko, EK Gaidamakova, VY Matrosova, A Vasilenko, A Venkateswaran, M Zhai, HM Kostandarithes, H Brim, KS Makarova, LP Wackett, JK Fredrickson, MJ Daly 2005. How radiation kills cells: survival of *Deinococcus radiodurans* and *Shewanella oneidensis* under oxidative stress. FEMS Microbiol Rev 29(2) 361-75.

Hanley JT, KK Foarde 2005 The use and selection of aerosol simulants at RTI. Defense Threat Reduction Agency, Joint Science Technology Office - Chemical and Biological Agent Simulant Summit Aug 10-11, 2005, Computer Science Corporation Executive Briefing Center, Falls Church, VA.

Hauer-Jensen M, LM Fink, J Wang 2004. Radiation and protein C pathway. Crit Care Med 32(5 Suppl): S325-30.

Hofacre, KC, S Burke 2005. Overview of battelle's CB simulant experience. Defense Threat Reduction Agency, Joint Science Technology Office - Chemical and Biological Agent Simulant Summit Aug 10-11, 2005, Computer Science Corporation Executive Briefing Center, Falls Church, VA.

Holman C 2002. Chemical and biological defense test and evaluation paradigm. Army Chief of Staff Weekly Summary 25 Nov 2002, 23-24.

Holman, CE, R Berkowitz, G Winslow 2007a. Technical Report of the effect of the Field of Regard (FOR) on the detector performance of the Joint Service Lightweight Standoff Chemical Agent Detector (JSLSCAD) Incr 1 (S). US Army AEC Report Alexandria VA.

Holman, CE, L Cash, T Lloyd, A Khan, C Pritts, K Blaylock, T Denning, L Seed, R Berkowitz, G Winslow 2007b. System Evaluation Report update for the Joint Service Lightweight Standoff Chemical Agent Detector Increment 1 (JSLSCAD Incr 1). US Army ATEC Report Alexandria VA.

Holman, CE, I Kuria, R. Berkowitz 2006a. A critical environmental factor in the test and evaluation of the Joint Service Lightweight Standoff Chemical Agent Detector (JSLSCAD). MORS

Holman C, I Kuria, N Dunn, J Timmerman, S Colegrove, P Serguievski, J Kleimeyer, R Altenbaugh, R Burkowitz, L Mcglynn, G Winslow 2006b. Report of the modeling and Simulation of the Joint Service Lightweight Standoff Chemical Agent Detector (JSLSCAD) (S). US Army Test and Evaluation publication Dec 2006, 200 pages.

Holman C, M Navarro, C Russell, C Jennings, D Anderson 2006c. System evaluation plan for the WSLAT methodology development program. US Army Test and Evaluation publication Oct 2006, 78 pages

Holman, C, A Parks, O Pozda, K Rose, A Benware, L Cash, D Hildebrant, A Khan, S Abdalla, C Pritts, M Smith, L Wollenberg, L Lawrence 2007c System evaluation report Joint Chemical Agent Detector (JCAD) Increment 1 (S). US Army ATEC Report Alexandria VA.

Holman C, CT Russell, C Jennings 2004. Extrapolating testing for biological warfare agents from the laboratory to a field environment. 20-22 Oct 2004 Tenth US Army Conference on Applied Statistics, Georgia Tech Hotel and Conference Center, Atlanta, GA.

Holman, CE, CM Sleeper 1996a. Technical note test and evaluation of the Biological Integrated Detection System (BIDS). US Army ATEC Report Alexandria VA.

Holman, CE, C Sleeper. 1996b. Test and evaluation of the biological detector system. Proceedings of the Army Operations Research Symposium. US Army ATEC Report Alexandria VA.

Holman, CE, G Winslow, R Burkowitz 2008 Evaluation of the Joint Service Lightweight Standoff Chemical Agent Detector (JSLSCAD), MORSS, US Coast Guard Academy, New London, CT

Holman C, G Winslow 2006 Technical note on the Lightweight Standoff Chemical Agent Detector, Increment 1 (JSLSCAD, Incr1) detection performance in tactical environments (S). US Army AEC Report Alexandria VA.

Hosmer DW, S Lemeshow 1989 Applied logistic regression. John Wiley and Sons, NY, 307 pages.

Jablonski, RE, WP Ashman 2005. US Army Edgewood Chemical and Biological Center: chemical and biological agent simulant knowledge base. Defense Threat Reduction Agency, Joint Science Technology Office - Chemical and Biological Agent Simulant Summit Aug 10-11, 2005, Computer Science Corporation Executive Briefing Center, Falls Church, VA.

Kaufman J 2005. Simulant summit. Defense Threat Reduction Agency, Joint Science Technology Office - Chemical and Biological Agent Simulant Summit Aug 10-11, 2005, Computer Science Corporation Executive Briefing Center, Falls Church, VA.

Kaufman J 2007. Physical science and technology division threat agent science capability area simulant development. July 10, 2007 TECMET, Huntsville, AL.

Kortepeter M, G Christopher, T Cieslak, R Culpepper, R Darling, J Pavlin, J Rowe, S Scott. 2001 USAMRIID's Medical management of biological casualties handbook. Maryland, USAMRIID. 165 p.

Krozga, M and C Holman. 1995. Biological Integrated Detection System (BIDS) limited user II Test and Evaluation Plan (TEP). US Army ATEC Report Alexandria VA.

Krozga, M and CE Holman. 1996. Biological Integrated Detection System (BIDS) limited user II Test and Evaluation Report (TER). US Army ATEC Report Alexandria VA

Kuria, I, L Seed, A Parks, J Chipman, A Khan, J Giese, C Pritts, C. Holman, J Timmerman 2006. System evaluation report of the Joint Service Lightweight Standoff Chemical Agent Detector. US Army ATEC Report Alexandria VA.

Mahle, J, ML Parham, A Balboa 2005. Simulant selection. Defense Threat Reduction Agency, Joint Science Technology Office - Chemical and Biological Agent Simulant Summit Aug 10-11, 2005, Computer Science Corporation Executive Briefing Center, Falls Church, VA.

Marchetti F, MA Coleman, IM Jones, AJ Wyrobek. 2006. Candidate protein biodosimeters of human exposure to ionizing radiation. Int J Radiat Biol. 82(9):605-39.

McBride MT, S Gammon, M Pitesky, TW O'Brien, T Smith, J Aldrich, RG Langlois, B Coslston, and KS Venkateswaran 2003. Multiplexed liquid arrays for simultaneous detection of simulants of biological warfare agents. Anal Chem. 75: 1924-30.

Musgrave, D and C Holman. 1997. Biological Integrated Detection System (BIDS) pre planned product improvement program IOTE test and System Evaluation Report Evaluation (SER). US Army ATEC Report Alexandria VA.

Musgrave, D, C Holman, S Tackett, K Finanger, J Fuller, R Jernigan, T Hillard, C Sleeper 2000 System Evaluation Report (SER) for the Biological Integrated Detection System (BIDS) Pre-Planned Product Improvement (P3I) System Evaluation Report (SER) US Army Test and Evaluation publication May 2000, 33 pages.

Palya, F 2005. Chemical Warfare Agent (CWA) simulant project. Defense Threat Reduction Agency, Joint Science Technology Office - Chemical and Biological Agent Simulant Summit Aug 10-11, 2005, Computer Science Corporation Executive Briefing Center, Falls Church, VA.

Peck, WA 2002. Joint Biological Point Detection System operational assessment 2 report. FR – 01-029, Air Force Operational Test and Evaluation Center Kirtland Air Force Base, New Mexico, 218 pages.

Radel, R 2005a. Dugway Proving Ground – Simulant Summit. Defense Threat Reduction Agency, Joint Science Technology Office - Chemical and Biological Agent Simulant Summit Aug 10-11, 2005, Computer Science Corporation Executive Briefing Center, Falls Church, VA.

Radel, R 2005b. National chemical and biological test and evaluation strategies – The TECMIPT. Defense Threat Reduction Agency, Joint Science Technology Office - Chemical and Biological Agent Simulant Summit Aug 10-11, 2005, Computer Science Corporation Executive Briefing Center, Falls Church, VA.

Romero, C. 2006. Product director, test equipment, strategy and support provides enhanced test capabilities. Chem-Bio Def Quart, 3 (3): 8-9.

Selman J 1983. Elements of radiobiology. Springfield, Illinois: Charles C. Thomas Publisher. 311 p.

Shepherd, S 2005. Simulants: decontamination. Defense Threat Reduction Agency, Joint Science Technology Office - Chemical and Biological Agent Simulant Summit Aug 10-11, 2005, Computer Science Corporation Executive Briefing Center, Falls Church, VA.

Shirakawa, K, CT Russell, CE Holman 2008 Estimating performance of a standoff biological detector system agents actual biological warfare agent. MORSS, US Coast Guard Academy, New London, CT

Song L, S Ahn, and DR Walt 2006. Fiber-optic microsphere-based arrays for multiplexed biological warfare agent detection. Anal Chem 78: 1023-33.

Tevault DE 2005. ECBC simulant efforts. Defense Threat Reduction Agency, Joint Science Technology Office - Chemical and Biological Agent Simulant Summit Aug 10-11, 2005, Computer Science Corporation Executive Briefing Center, Falls Church, VA.

Thermos, JN, DO Andersen, AJ Mohr, and BS Williams 2000. Abbreviated test report for the Production Qualification Test (PQT) of the Joint Biological Point Detection System (JBPDS). WDTC-TR-00-075, West Desert Test Center, Dugway, UT, 109 pages.

Thermos, JN, SL Schanaman, DO Andersen, BS Williams, D Blodgett, T Spackman 2001. Abbreviated test report for the mini field trials 2 of the Joint Biological Point Detection System. WDTC-TR-01-090, West Desert Test Center, Dugway, UT, 69 pages.

Thermos, JN, SL Schanaman, BS Williams 2002a. Abbreviated test report for the operational assessment 2 of the Joint Biological Point Detection System. WDTC-TR-01-133, West Desert Test Center, Dugway, UT, 78 pages.

Thermos, JN, SL Schanaman, and BS Williams 2002b. Abbreviated test report for the biological aerosol warning sensor test in the ambient breeze tunnel. WDTC-TR-02-042, West Desert Test Center, Dugway, UT, 40 pages.

Thomas JH, SK Kim, PJ Hesketh, HB Halsall, and WR Heineman 2004. Beab-based electrochemical immunoassay for bacteriophage MS2. Anal Chem. 76: 2700-7.

Touchton, J 2005. Validation and accreditation of threat representation for test and evaluation. Defense Threat Reduction Agency, Joint Science Technology Office - Chemical and Biological Agent Simulant Summit Aug 10-11, 2005, Computer Science Corporation Executive Briefing Center, Falls Church, VA.

Tracy, D, B Burkholder, and W Brence 2005. Shifting the agent-simulant paradigm. Defense Threat Reduction Agency, Joint Science Technology Office - Chemical and Biological Agent Simulant Summit Aug 10-11, 2005, Computer Science Corporation Executive Briefing Center, Falls Church, VA.

Weinstein RS, K Alibek 2003. Biological and chemical terrorism. New York: Thieme. 161 p.

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